Proceedings

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AMERICAN ACADEMY OF FORENSIC SCIENCES
410 North 21st Street
Colorado Springs, CO 80904
Phone: (719) 636-1100
Fax: (719) 636-1993
E-mail: membship@aafs.org
Website: www.aafs.org

PROCEEDINGS

of the American Academy of Forensic Sciences 68th Annual Scientific Meeting

The Proceedings of the American Academy of Forensic Sciences is an official publication of the American Academy of Forensic Sciences (AAFS). It is devoted to the publication of the abstracts of technical oral papers and posters presented at the AAFS annual scientific meeting. These include various branches of the forensic sciences such as pathology, toxicology, anthropology, psychiatry, immunology, odontology, jurisprudence, criminalistics, questioned documents, digital evidence, and engineering. Similar submissions dealing with forensic oriented aspects of the social sciences are also included.

Please note that some of the abstracts included in the Proceedings deal with topics, results, and/or conclusions which are controversial. The publication of abstracts does not imply that the AAFS, its sections, or the individual section program chairs/committee members have verified or agree with the studies, results, and/or conclusions of each abstract. During the process of planning a scientific program, it is impossible to "peer-review" each abstract and presentation to the degree that is accomplished during manuscript review. Abstracts and presentations are accepted, in part, so that they can be critiqued and reviewed by other scientists. Thus, a forum is created to discuss controversial issues.

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English is the official language of the AAFS and its meetings; neither oral nor written translations will be provided.

SPECIAL SESSIONS

S1 Innovative Science — How Advances in Technology Transform Forensic Science

Jeri D. Ropero-Miller, PhD, RTI International, 3040 Cornwallis Road, PO Box 12194, Bldg 7, Rm 211, Research Triangle Park, NC 27709; Marla E. Carroll, BS, Forensic Video & Audio Assoc, 6919 W Broward Boulevard, Ste 222, Plantation, FL 33317; Nancy Rodriguez, PhD*, National Institute of Justice, Dept of Justice/Office of Justice Programs, 810 Seventh Street, NW, Washington, DC 20531; Kenneth G. Furton, PhD*, Florida International University, International Forensic Research Institute, University Park, Miami, FL 33199; Jed S. Rakoff, JD*, US District Court, Southern District NY, 500 Pearl Street, New York, NY 10007-1312; John Collins, Jr., MA*, The Forensic Foundations Group, PO Box 227, Dewitt, MI 48820; Richard A. Guerrieri, MS*, 1 Corduroy Court, Stafford, VA 22554; Kurt B. Nolte, MD*, Office of Medical Investigator, MSC07 4040, 1 University of NM, Albuquerque, NM 87131-0001; Christina G. Hayes, BS*, St. Louis Metropolitan Police Department, 1915 Olive Street, St. Louis, MO 63103; Amanda R. Hale, MA*, North Carolina State University, 127 David Clark Labs, Campus Box 7617, Raleigh, NC 27695; and Zeno J. Geradts, PhD*, Netherlands Forensic Institute, Laan van Ypenburg 6, Den Haag, SH 2497 GB, NETHERLANDS*

After attending this presentation, attendees will better understand some best practices for technology adoption and implementation that have improved efficiency, quality, accuracy, reliability, and operational excellence in forensic sciences and beyond. Furthermore, the Interdisciplinary Symposium will help attendees understand the benefits and risks of emerging technologies to enable consideration and implementation.

This presentation will impact the forensic science community by highlighting how technology and its adoption can advance teaching and learning experiences, improve operational and legal standards, and embrace scientific innovation.

Whether at the scene of a death or a crime, in the forensic laboratory, or in the courtroom, technologies are used every day by practitioners to impart evidentiary proof and thereby solve cases. Forensic science has always been held to a high standard in order to uphold justice and for this to continue, forensic science must evolve and innovate.

As science progresses, answering a research question utilizing evidence-based science and technology typically leads to more questions. In fact, scientific knowledge begets new technologies, which beget new observations and scientific knowledge, which begets the next technological advancement. Keeping up with these advances in a forensic environment requires change, both operationally and culturally. Understanding, embracing, communicating, and when necessary, enforcing these changes requires the involvement of all stakeholders — the government, the criminal justice system, medical and forensic communities, and the public.

The 2016 American Academy of Forensic Sciences Interdisciplinary Symposium program will help forensic scientists keep pace with technology-enabled opportunities by highlighting advances in forensic science that have improved efficiency, quality, accuracy, reliability, and operational excellence. From forensic science disciplines including pattern comparison and forensic medicine to newer ones such as digital and multimedia sciences and next generation sequencing, innovation in science and technology is all about understanding what the technology brings to the science and how the science can harness new knowledge and information to improve impact and confidence.

This Interdisciplinary Symposium program will include prominent speakers who support technology adoption in academia, the government and private sectors, management, and the legal system. This program will continue with innovative and emerging technology "stars" among us and "integrators" of technology who will share how they have experienced and continue to transform their practice based on the latest technology.

Nancy Rodriguez, PhD

Keynote Presentation

As the federal government's lead agency for forensic science research and development, as well as the administration of programs to facilitate technical assistance, the National Institute of Justice (NIJ) has a prominent role in directing efforts to address the needs of the forensic science community. Using various sources such as the Report issued by the National Academy of Sciences (NAS) in 2009 — Strengthening Forensic Science in the United States: A Path Forward — NIJ has made an unprecedented investment to help strengthen forensic science in the United States. The NIJ remains committed to a strategy that couples rigorous research and development with technical assistance to serve the forensic science community. This approach provides the forensic science field with evidence-based research to create long-term success and ultimately improve public safety.

Kenneth G. Furton, PhD

Historically, higher education has been focused on disseminating knowledge and creating new knowledge, but increasingly, universities are becoming hubs for innovation and entrepreneurship and helping to drive the economic development

of the communities they serve. This trend can be transformative for forensic science as the translational research occurring in major academic forensic programs is spurring advances in many fields of forensic science that will impact the courtroom as well as the corporate boardroom. This presentation will highlight how academic forensic scientists have and will transform the field of forensic science in a variety of areas with a focus on detection science where trace detection of evidence and odors left from removed evidence is having a major impact on forensic science.

Jed S. Rakoff, JD

In the Anglo-American legal system, change tends to be incremental, with judges attempting to fit new situations and advances into the framework of previously developed legal principles; however, when it comes to technological advances, judges who rarely have much technological training or knowledge often find this difficult to do. This presentation will examine some of the difficulties judges have faced in dealing with technological advances in the forensic sciences and will suggest ways some of the problems of translating these advances into useable legal form might be better approached.

John M. Collins, Jr., MA

When forensic science professionals think of technology, their attention understandably gravitates toward innovations that relate directly to casework and the testing of evidence. But technology is not only about scientific practice. Managerial technology and innovations dealing with the administration of forensic science organizations are equally important. In this session, attendees will be introduced to the concept of administrative technologies and the way innovation can improve how forensic science organizations are managed. By examining some best-in-class practices from both inside and outside the forensic sciences, attendees will come to appreciate how technology can be leveraged in forensic science, not just for the testing of evidence, but in the management of people, customers, and organizational cultures.

Richard A. Guerrieri, MS

Forensic DNA analysis through Capillary Electrophoresis (CE) -based typing of Short Tandem Repeats (STR) is a well-established and successful technology with widespread technical acceptance. The emergence of Next Generation Sequencing (NGS) introduces opportunities for enhanced discrimination within mixtures and human remains, as well as identity, physical appearance, and ancestry relationships. NGS also introduces levels of change which are disruptive to present forensic laboratory approaches and will require modifications of established quality assurance practices and the development of new measures. NGS experiences in this area will be shared and implementation strategies for consideration by the forensic DNA community will also be discussed.

Kurt B. Nolte, MD

Advanced radiologic imaging modalities such as Computed Tomography (CT) scanners are transforming the practice of forensic pathology. CT allows for the rapid acquisition of a full volume of morphologic data that can be reconstructed in multiple planes as well as 3D perspectives. These images are detailed and can cover the full body. Research performed at the New Mexico Office of the Medical Investigator (OMI) has demonstrated that while both CT and autopsy have limitations in recognizing disease and injuries, they can be complementary in achieving the fullest diagnostic data set. This research has also demonstrated that in certain decedent cohorts, CT can supplant autopsy by developing an adequate diagnostic data set for accurately determining the cause of death. The OMI CT scanner is used daily by forensic pathologists to triage cases and to supplement and supplant autopsy.

Christina G. Hayes, BS

In the world of chemistry, there is a vast array of instrumentation that is available for use, yet in forensic drug chemistry, generally only a few instruments are utilized. By exploring the new technology available and comparing it to the standard instrumentation used with specific groups of drugs, it is possible to expand the drug chemists' repertoire for drug analysis.

Amanda R. Hale, MA

Digital imaging innovation is integral to advancing methods in forensic anthropology. The application of imaging techniques such as Computed Tomography (CT), 3D laser scanning, and digitization has already increased accuracy when performing putative identifications, ancestry estimation, and juvenile aging. In addition, digital imaging has increased database reference material used for both research and application. In combination with advanced statistical techniques, these provide a powerful new avenue for developing more precise methods in skeletal biology.

Zeno J. Gerdts, PhD

The development of digital and multimedia sciences is rapid due to the growth of data and the wide range of devices where digital evidence can be found; smartphones and most electronic devices now have digital storage that communicates with networks. Several sources state that 90% of the digital data has been produced during the last two years. Due to these rapid developments of big data, new techniques can be used and validation is crucial. Several developments in facial and image recognition based on deep learning algorithms have seen good progress and can be used in practice to assist forensic casework. New techniques on weak signal analysis will cause more possibilities for predictive methods. Also, if data is not accessible due to encryption, techniques for analyzing data streams can also help in cyber forensics cases.

S2 Viva La Forensics

Lara Frame-Newell, MA, Office of the Chief Medical Examiner, 400 E Jackson Street, Richmond, VA 23219; Sarah J. Ellis, MS*, North Carolina State Crime Laboratory, 121 E Tryon Road, Raleigh, NC 27603; Amanda R. Hale, MA*, North Carolina State University, 127 David Clark Labs, Campus Box 7617, Raleigh, NC 27695; Lindsay Saylors, 6258 W 60th Street, Chicago, IL 60638; Betzaida L. Maldonado, MSFS, 6215 Denmeade Drive, Atlanta, GA 30345; Jeremy M. Manheim, 2387 Shaker Lane, Apt F, Lebanon, IN 46052; Alicja K. Lanfear, PhD, Middle Tennessee State University, Dept of Biology, Box 60, Murfreesboro, TN 37132; Christina G. Hayes, BS*, St. Louis Metropolitan Police Department, 1915 Olive Street, St. Louis, MO 63103; Kelsey A. Carpenter, BS*, Unlisted, Howell, MI ; Brianna B. Bermudez, BS, 2297 Knob Hill Drive, Apt 14, Okemos, MI 48864; Jacob Griffin, BS, 16665 Danville Road, Danville, IA 52623; Ja'Neisha Hutley, MS*, 1500 Locust Street, Apt 1704, Philadelphia, PA 19102; John Nixon, CEng, MBA*, ARC, PO Box 66, Bippus, IN 46713; Raymond G. Miller, DDS*, 122 Covington Road, Buffalo, NY 14216; Gary M. Berman, DDS*, 9840 Haggerty Road, Belleville, MI 48111; John A. Williams, PhD*, Western Carolina University, Anthropology and Sociology, 101 McKee Hall, Cullowhee, NC 28723; Joseph Almog, PhD*, Hebrew University, Casali Inst of Applied Chem, Jerusalem 91904, ISRAEL; Joan A. Bytheway, PhD*, Sam Houston State University, College of Criminal Justice, Box 2296, Huntsville, TX 77341-2296; Helmut G. Brosz, BASc, PEng*, Brosz Forensic Services, 64 Bullock Drive, Markham, ON L3P 3P2, CANADA; Linton Mohammed, PhD*, Forensic Science Consultants, Inc, 433 Airport Boulevard, Ste 406, Burlingame, CA 94010-2014; Alan A. Price, MA*, University of Northern Colorado, Candelaria Hall, Rm 2285, Campus Box 147, Greeley, CO 80639; Nikolas P. Lemos, PhD*, OCME, Forensic Lab Division, Hall of Justice, N Terrace, 850 Bryant Street, San Francisco, CA 94103; J.C. Upshaw Downs, MD*, GBI ME, 925 A Mohawk Drive, Savannah, GA 31419; Claire E. Shepard, MS*, La Delta Community College, 7500 Millhaven Road, Monroe, LA 71203; Federica Collini, MD*, Via Mangiagalli 37, Milan 20133, ITALY; Noelle J. Umback, PhD*, OCME, Dept of Forensic Biology, 421 E 26 Street, New York, NY 10016; and Cheryl D. Hunter*, 403 Pioneer Creek Drive, Florissant, CO 80816*

After attending this presentation, attendees will have a better understanding of casework and solving cold cases within the fields of forensic science. Additionally, attendees will better understand how to create a resume and how to apply and interview for a job.

This presentation will impact the forensic science community by demonstrating cases where forensic science was key to case resolution. This will show attendees what real casework is and how real-life cases are solved. Cases will be presented from beginning to end.

Each year at the American Academy of Forensic Sciences (AAFS) Annual Scientific Meeting, the Young Forensic Scientists Forum (YFSF) provides a program for students and forensic scientists with less than five years of professional experience. The session allows attendees to interact with peers as well as with the professional speakers and to build professional relationships that foster growth and mentorship opportunities. Special session topics provide attendees with a broad overview of the many opportunities in the field of forensic science. In addition to the special session, the YFSF session offers two opportunities for young forensic scientists to present their own work or research: the YFSF Bring Your Own Posters (BYOP) Session and the YFSF Bring Your Own Slides (BYOS) Session. The Forensic Sciences Foundation (FSF) Emerging Forensic Scientists Award winner is also invited to present her award winning paper during this special session.

For the AAFS 68th Annual Scientific Meeting in Las Vegas, NV, the YFSF Special Session will present *Viva La Forensics!* The special session will be held on Tuesday, February 23, 2016, and will include speakers from many of the AAFS sections who will discuss cases where forensic science was key to solving the case. Through the presentations, attendees will learn how forensic science can be used to change the outcome of a case. Attendees will be exposed to the real life of a forensic scientist and to actual witness testimony.

Following the Tuesday session, the YFSF BYOP Session will be presented in the evening, giving young professionals the opportunity to showcase current cases and research being worked on in a poster format.

The annual YFSF BYOS Session takes place the evening of Wednesday, February 24, 2016, and will include presentations from students and young professionals. YFSF does not require presenters of YFSF BYOS and BYOP Sessions to be members of AAFS and does not require they attend the special session, but it is encouraged that they do so. The program will conclude on Thursday, February 25, 2016, with the annual YFSF Breakfast Session which includes a résumé review panel. Attendees of the breakfast session must be registered for the YFSF Special Session.

As is the tradition, the YFSF Breakfast Session focuses on developing professional skills for the next generation; however, this year will be a little different. Instead of planned speakers, members of various AAFS sections have been asked to participate in a Q&A Panel to help facilitate conversation between young professionals and professionals already established in their field. After the panel, attendees will have the opportunity to receive résumé assistance and feedback from AAFS members.

The special session provides students, young professionals, and AAFS members with a way to foster career-long relationships. The main goal of the YFSF is to encourage mentorship between young and veteran forensic scientists. Attendees are encouraged to apply for membership in the AAFS and are given guidance on the many opportunities available to aid in career enrichment.

*Presenting Author

BREAKFAST SESSIONS

BS1 Death in a Bathtub: The Defense of Drew Peterson

Jeffrey M. Jentzen, MD, University of Michigan, 300 N Ingalls, NI2D19 - SPC 5452, Ann Arbor, MI 48109; and Mary E.S. Case, MD*, 6059 N Hanley Road, St. Louis, MO 63134*

After attending this presentation, participants will better understand the courtroom procedures for admission of evidence and expert witness testimony. In addition, attendees will learn the factors involved in injury identification and analysis with an emphasis on the investigation of drowning.

This presentation will impact the forensic science community through the multidisciplinary reconstruction of one of the most riveting cases in recent American trial history. The presenters will detail the factors and evidence that influenced their decision process and assist future prosecutors, judges, and death investigators in the courtroom procedures.

In 2004, the body of Kathleen Savio, the third wife of policeman Drew Peterson, was found dead in the bathroom of her suburban Chicago home. Her body was found lying in an empty bathtub with a small laceration to the left back of the scalp. Froth oozed from her nostrils. There were some bruises to her left side. Toxicology analysis was negative for intoxicating drugs and alcohol. The initial investigation concluded that the death was the result of drowning and the coroner certified the death accidental. In 2007, Peterson's fourth wife, Stacey Peterson, disappeared — her body was never recovered. In light of Stacey Peterson's disappearance, authorities re-opened the investigation into Savio's death. Savio's body was disinterred in 2007 and re-examined in two separate autopsies performed by a group of forensic pathologists. The pathologist identified areas of hemorrhage over the left hip region, not appreciated at the initial examination. In light of the additional evidence, the experts concluded that Savio's death was a homicide.

In criminal cases, there is a constitutional dimension to hearsay. The Sixth Amendment gives criminal defendants the right to confront witnesses; since a hearsay statement is made out of court, there is no opportunity for the defendant's criminal defense attorney to cross-examine the witness, and thus no confrontation. This means hearsay statements are harder to get into evidence even via the traditional hearsay exceptions when they are used against a criminal defendant.

Prosecutors collecting evidence identified the fact that Stacey Peterson had confided with family and friends implicating her husband, Drew Peterson, as her murderer. Unable to question the dead witness, Stacey, prosecutors petitioned the Illinois legislature to create a new exemption to the hearsay rule, which became known as "Drew's Law." The law allowed for the admission of evidence in cases where the witness was not available to testify due to the actions of the defendant. Meanwhile, defense experts unsuccessfully attempted to exclude testimony related to Stacy Peterson's disappearance in a 2010 evidentiary trial.

The trial into the death of Kathleen Savio began in August of 2012. For more than six weeks of grueling testimony, the media provided the day-to-day revelations of the case. Five forensic pathologists testified in the case that called into question the cause and manner of death. All the pathologists agreed that Savio died of drowning. The pathology testimony rested on questions of the pathological findings of concussion, postmortem artifacts, orientation of injuries, and causes of accidental drowning.

In light of new legislation, the prosecution was allowed to present incriminating verbal testimony against Drew Peterson. Peterson was eventually convicted and sentenced to 38 years in prison for his role in death of Kathleen Savio. The jurors said that the most convincing testimony was the hearsay statements allowed into evidence under the new "Drew's Law." Prosecutors successfully fought to have statements made by Stacy Peterson and Savio to acquaintances admitted into evidence. In February 2013, the defense was denied a new trial. The trial left numerous questions unanswered and created a precedent of allowing indefensible hearsay testimony.

This presentation will provide courtroom presentation of evidence, expert testimony, the role of the medical witnesses, and criminalistic's evaluation in the trail of Drew Peterson. Participation of attendees is encouraged and will bring to life the tension of the courtroom in this precedent-setting prosecution.

Drew Peterson, Bathtub Drowning, Drew's Law

BS2 Death From a Distance: The Etiology of Serial Sniper Homicides

Robert J. Morton, MS, Fauquier County Sheriff's Office, 78 Lee Street, Warrenton, VA 20186; and Mary B. Collins-Morton, MS*, FBI Academy, NCAVC BAU4, Quantico, VA 22135*

After attending this presentation, attendees will better understand the unusual nature of serial sniper homicides and the difficulties faced by law enforcement, forensic specialists, and prosecutors in dealing with these cases.

This presentation will impact the forensic science community by highlighting the etiology of serial sniper homicides, the problems involved in determining case linkage between the different shootings, the circumstantial nature of the forensic evidence, and the difficulties involved in the prosecution of these infrequent crimes.

This presentation is designed to provide a historic overview of serial sniper homicides within the context of murder in general and serial murder, specifically. This presentation will also provide the statistical instances of serial sniper homicides compared to other types of murders as well as outline the investigative and forensic difficulties faced by law enforcement and the forensic community when dealing with one of these rare cases. Additionally, an extensive case study will be presented regarding a serial sniper case that took more than ten years to successfully convict the offender.

The Federal Bureau of Investigation's (FBI's) National Center for the Analysis of Violent Crime (NCAVC) is routinely consulted by federal, state, and local authorities in a variety of bizarre and repetitive violent crimes, especially homicides. NCAVC assistance was requested by local authorities regarding the case of a serial sniper. The four shooting incidents occurred during a highly contested divorce proceeding involving the offender. The first and second shootings were directed at the lawyer representing the offender's wife and the judge presiding over the divorce hearing, both of whom were shot at while inside their residences. Both were uninjured. The lawyer representing the offender's wife was shot at a second time while in his law office. The bullet struck him in the left eye, causing the loss of the eye; however, he survived the attack. The last attack occurred seven years later and targeted the male coworker with whom the offender's wife had previously had an affair. The victim, who was now a married father of three, was shot and killed in the yard of his residence. The laboratory determined the recovered bullet fragments from the shootings were consistent with a .22 caliber centerfire bullet and were fired through a similar rifle.

The investigation quickly focused on the offender; however, a series of search warrants failed to locate the specific rifle or similar ammunition involved in the attacks. The lack of evidence emboldened the offender and he began a public campaign professing his innocence and crusading against the "corrupt" criminal justice system. After one of the search warrants had been served, the offender placed a written response on the front porch of the judge who had signed the warrant, even though he lived in a different jurisdiction located more than an hour away. Additionally, the offender became active in the local political party and attempted to exert pressure on the local authorities to stifle the investigation.

Based upon the highly charged nature of this case, the "fear factor" generated by the shootings, the defiant demeanor displayed by the offender, and the lack of direct evidence, the police department requested assistance from the FBI. Both the local FBI office and the NCVAC provided assistance. During the joint consultation with the NCAVC, it was decided to convene a grand jury to address the four shootings. The strategy involved calling the offender as the first witness and outlining the legal ramifications for contacting and/or intimidating other witnesses testifying before the grand jury. The offender subsequently interfered with several witnesses, was charged and convicted of four counts of witness tampering, and was given a 21-year sentence. He was also indicted on numerous charges for the four sniper shootings, including murder and attempted murder.

The complex trial lasted more than two months. While there was no direct forensic evidence linking the offender, a variety of circumstantial evidence was presented outlining the unusual nature of the series of shootings, the relationship of the victims who were targeted, the offender's skill with weapons, and the various statements made by the offender. During the trial, numerous witnesses were called including forensic experts, investigators, the shooting victims, the offender's ex-wife, a member of the NCAVC, and a police department Special Weapons And Tactics (SWAT) sniper. The jury found the offender guilty of 31 separate counts and sentenced him to a life sentence plus 288 years.

This case highlights the complex nature of sniper murders, the value of circumstantial evidence, and the benefit of forensic experts, investigators, subject-matter experts, and prosecutors working cooperatively.

Serial Sniper, Serial Murder, Sniper

BS3 A Primer on the Structure and Activity of the National Institute of Standards and Technology's (NIST's) Organization of Scientific Area Committees (OSAC)

Barry K. Logan, PhD, NMS Labs/CFSRE, 3701 Welsh Road, Willow Grove, PA 19090; Mark D. Stolorow, MS, MBA*, NIST Special Programs Office, Organization of Scientific Area Committees, 100 Bureau Drive, MS 8102, Gaithersburg, MD 20899-8102; Sally S. Aiken, MD*, 5901 N Lidgerwood, Ste 24B, Spokane, WA 99208; Marc A. LeBeau, PhD*, FBI Laboratory, 2501 Investigation Parkway, Quantico, VA 22135; Gregory G. Davis, MD*, Jefferson County MEO, 1515 6th Avenue S, Rm 220, Birmingham, AL 35233-1601; and Christian G. Westring, PhD*, 2300 Stratford Avenue, Willow Grove, PA 19090*

After attending this presentation, participants will be able to discuss the structure and activities of the various committees and subcommittees that comply with the new NIST OSAC process established to develop standards and guidelines for the professional practice of forensic science.

This presentation will impact the forensic science community by introducing the OSAC process, its accomplishments, and its plans to the forensic science community, AAFS members, and meeting attendees. Information will be presented to assist attendees in becoming involved with and contributing to the process.

This presentation is designed to provide a brief introduction to the new and important structure created and driven by the forensic science community in partnership with NIST to develop standards and guidelines for professional practice of forensic science. If you have heard about this new organization and its assignment but want to better understand how it works, its potential to create systemic change in the forensic sciences, and opportunities for everyone to be involved; this is the session for you.

In 2013, NIST, in consultation with the Department of Justice (DOJ), began the process of seeking input from the forensic science community to create a registry of standards that represented the priorities and consensus of the practitioners of forensic science, but that was also subject to review, scrutiny, and input from other stakeholders including the criminal justice community (lawyers and judges), researchers, statisticians, and the general public. The intent was to create an open, transparent, and accountable process that would reduce the risk of bad science being used in the courtroom and create a means for more consistent practice and continuous improvement in the application of scientific methods and practice to criminal investigations.

In 2014, NIST created the OSAC to take on this task and began recruiting members. The organization is structured with a governing board, the Forensic Science Standards Board (FSSB), and served by three resource committees (Human Factors, Legal Resources, and Quality Infrastructure) and five scientific area committees (Biology/DNA, Chemistry/Instrumental Analysis, Crime Scene/Death Investigation, Digital/Multimedia, and Physics/Pattern Interpretation) to manage and support the work of the subcommittees, and the subcommittees themselves. The OSAC currently has 24 subcommittees (enumerated on NIST's web site) which either replaced or augmented the previous Scientific Working Group's (SWG's) standards development activities. The subcommittees and their derivative task groups work on the creation of new or adoption of existing United States or international standards, developed in a manner consistent with the widely recognized ANSI standards development process, subject to public comment, and publication in OSAC's approved standards and guidelines registries.

The process has been enthusiastically adopted by the forensic science community and, as of August 2015, standards were already in development for submission to the review and adoption procedure. In July 2015, the AAFS announced its intent to become an ANSI approved Standards Development Organization (SDO) and to take a leadership role in support of this new process.

Although there are many OSAC events which take place during the AAFS meeting, this presentation is designed to act as a primer to AAFS members and meeting attendees wanting to quickly get up to speed with the new process and the organization. The presentation will feature brief discussions from members of the various levels of the OSAC organization who will describe their roles and their progress to date and answer questions about how to become involved in this critical new process in support of the future and continued professionalization of forensic science.

OSAC, NIST, Forensic Science Standards

BS4 One Night in August: The I-35W Bridge Collapse in Minneapolis

Andrew M. Baker, MD, Hennepin County ME, 530 Chicago Avenue, Minneapolis, MN 55415; and Owen L. Middleton, MD, Hennepin County ME, 530 Chicago Avenue, Minneapolis, MN 55415*

After attending this presentation, attendees will understand the role of, the challenges posed to, and the lessons learned by the medical examiner in a high-profile, multi-fatality mass disaster.

This presentation will impact the forensic science community by providing deeper insights into the role of the medical examiner and forensic pathologist in managing a mass fatality incident. Special attention will be paid to techniques for identifying remains, communicating with families, informing the public, and working with other agencies and elected leaders.

On August 1, 2007, during the height of rush hour, the eight-lane I-35W Bridge in Minneapolis collapsed, sending scores of vehicles into the Mississippi River.

Bridge construction started in 1964 at a cost of ~ $5.2 million and the bridge opened in 1967. Originally striped for four lanes with an expected use of 66,000 vehicles per day, the bridge was restriped to eight lanes in 1988. At the time of the collapse, the 14-span, 1,907 feet long bridge carried an estimated 141,000 vehicles per day.

The medical examiner's office was one of some 75 city, county, state, federal, and private organizations that were eventually involved in the recovery of bodies and investigation of the collapse. Water visibility, current speed, biohazards, and steel and concrete in the river made the recoveries of the victims difficult. The medical examiner's office worked with law enforcement agencies and dive teams to develop a protocol for handling victims' remains with as much dignity and privacy as possible, given the challenges of the recoveries and the intense media scrutiny. Medical examiner investigators proactively contacted the families of the missing to obtain as much antemortem identifying material as possible to facilitate victim identification when bodies were found. In all but one case, identifications and autopsies were completed, and remains released to the families, in less than one day following recovery.

The challenges of the disaster site led to an operation spanning approximately three weeks before the last victim was found. This presentation focuses on the role of the medical examiner in the days and weeks following the bridge collapse, with an emphasis on identification techniques; communications with families, the media, and elected leaders; a review of what did (and did not) go well; and a summary of lessons learned.

Bridge, Collapse, Mass Fatality

BS5 Back to the Future — A Journey Across the Timelines and Possible Realities for the Future of Forensic Sciences

J.C. Upshaw Downs, MD, GBI ME, 925 A Mohawk Drive, Savannah, GA 31419; and Carla Miller Noziglia, MS*, 305 Ascot Drive, Aiken, SC 29803-7833*

After attending this presentation, attendees will have a better understanding of the history of the forensic sciences, including important dates and events. Additionally, attendees will learn how different sequences of events may have led to vastly alternate realities — allowing for an informed discussion about how to best guide the future course of forensic sciences.

This presentation will impact the forensic science community by discussing important past dates and events and detailing how these events helped shape the present state of the forensic sciences. This knowledge will allow attendees to positively steer the future course of forensic science practice toward the best possible outcome.

Thirty years ago, a film about a time-traveling teenager and mad scientist explored the concept of parallel realities based on skewing past, present, and future timelines dependent on actions in the "then" present. In one journey to our present day (October 21, 2015), a radically different yet oddly familiar and plausible future greets the two partners in time. Unfortunately, the law of unintended consequences has led to a projected future with disastrous results, necessitating intervention in the past. Attempts to change events then lead to further future problems, requiring actions in the past to "correct" the altered future and… the rest, as they say, is history.

The history of forensic science dates to antiquity but a few key dates and events include 1194 (Articles of Eyre re-establishing the office of Coroner in the United Kingdom); 1248 (The Washing Away of Wrongs — medical investigation of death); 1609 (document examination); 1784 (physical matching); 1840 (arsenic poisoning); and 1888 (Jack the Ripper). The modern forensic era extends through Galton's Fingerprints in 1892. Academic forensic science can be traced to 1902 at the University of Lausanne, Switzerland. Fingerprint evidence arrived in the United States by way of the 1904 World's Fair, courtesy of Scotland Yard. Chief August Vollmer, credited by many as "the father of modern law enforcement" soon after (1907) created the first crime laboratories as part of the Berkley, California Police Department, an idea which expanded with his move to Los Angeles in 1923. The Bureau of Investigation (later FBI) created the first national forensic lab in 1926. State crime labs began to be developed in the early 1930s. The same decade saw criminalistics established as an academic discipline. Other developments ensued with the eventual creation of the American Academy of Forensic Sciences in 1948 and the National Association of Medical Examiners in 1966. Advances continued with the passage of time, including the creation of the Federal Rules of Evidence (1975), DNA application to forensics (1980s), National DNA Database (1994), National Commission on the Future of DNA Evidence (1998), Paul Coverdell National Forensic Science Improvement Act (1999), National Academy of Forensic Sciences Report — Strengthening Forensic Science in the United States: A Path Forward (2009), and National Commission on Forensic Science (2013).

Utilizing the time vehicle to consider past actions and consequent future timelines, attendees will be led through several key points: past, present, and future - in the forensic timeline, in order to illustrate where then-current thoughts and/or actions skew the chain of events leading the community astray to an undesired future. Recognizing that certain future consequences can be traced to intersections of significant persons and actions at critical points in time leads to the conclusion that strategy may allow a directed and desired future result. The ultimate goal is to stimulate consideration of alternatives and discussion about the most prudent course for present-day actions to assure the best possible future.

"…[Y]our future hasn't been written yet. No one's has. Your future is whatever you make it. So make it a good one…."[1]

Reference(s):

1. (Back to the Future, Part III) http://www.imdb.com/title/tt0099088/quotes

History, Forensic Science, Timeline

BS6 Thomas Krauss Memorial Bitemark Breakfast — Forensic Anthropology: Science Into Fiction

Kathleen J. Reichs, PhD, UNC - Charlotte, Dept of Anthropology, Charlotte, NC 28223; and Laura C. Fulginiti, PhD, Forensic Science Center, 701 W Jefferson, Phoenix, AZ 85007*

After attending this presentation, attendees will have a better understanding of the field of forensic anthropology and of the processes involved in creating fiction based on science. Emphasis will be on the writing of novels and screenplays.

This presentation will impact the forensic science community by providing an overview of the field of forensic anthropology and will describe techniques used in creating plausible fiction based on scientific procedures and principles.

Forensic anthropology is a sub-specialty within physical anthropology that combines knowledge of the human skeleton with skills in various areas of forensic protocol, including the recovery and analysis of modern human remains. A fully accredited forensic anthropologist will be certified by the American Board of Forensic Anthropology (ABFA). This requires achievement of a PhD, successful completion of a certification exam, adherence to a set of ethical standards, and regular reporting on continuing education requirements.

The forensic anthropologist analyzes compromised human remains — the decomposed, mummified, mutilated, burned, dismembered, and skeletal. She addresses questions of identity, manner of death, time since death, and, in some cases, postmortem body treatment.

The forensic anthropologist may function in any of a variety of contexts, including, but not limited to, medical examiner and coroner offices, government laboratories, disaster recovery teams, human rights efforts, law enforcement agencies, and the military. The forensic anthropologist does not operate in a vacuum but works with specialists in many other areas, including forensic odontology.

Over the past two decades, the analysis of crime scenes and crime victims has caught the attention of the general public. Forensic science has exploded onto the stage of pop culture, and practitioners have been portrayed in books and on the large and small screens. Dr. Temperance Brennan is the protagonist in 18 novels and the main character in the longest-running scripted drama in the history of the Fox network. Each of the Temperance Brennan books and the Young Adult (YA) Virals books and each episode of the television series Bones takes the reader or viewer into a context in which forensic investigators work. Each highlights a different area of expertise within the forensic sciences.

This presentation will discuss the process of fictionalizing forensic science by drawing upon the speaker's experience as a forensic anthropologist, a writer, and a television producer. The writing of a novel will be compared to the writing of a screenplay.

Anthropology, Novels, Screenplays

EVENING SESSION

ES1 The American Academy of Forensic Sciences (AAFS) Standards Development Process

Jennifer F. Limoges, MS, New York State Police, Forensic Investigation Center, 1220 Washington Avenue, Bldg 30, Albany, NY 12226-3000; Lucy A. Davis, BHS, LDH Consultants, 2944 N Mayo Trail, Pikeville, KY 41501; Mary C. McKiel, PhD*, The McKiel Group, LLC, 684 Southern Hills Drive, Arnold, MD 21012; Kenneth W. Aschheim, DDS, 44 E 67th Street, New York, NY 10065; Brad J. Wing, MS*, 4401 Chesapeake Street, NW, Washington, DC 20016-4423; and Teresa L. Ambrosius*, 410 North 21st Street, Colorado Springs, CO 80904*

After attending this presentation, attendees will understand the standards development process and how the AAFS Standards Development Organization (SDO) process will work.

This presentation will impact the forensic science community by educating attendees on how the AAFS will be involved in generating American National Standards for the forensic sciences.

The development of standards and guidelines for forensic science has become a priority within the criminal justice community. The National Commission of Forensic Sciences (NCFS) and the National Institute of Standards and Technology (NIST) Organization of Scientific Area Committees (OSAC) are actively pursuing this goal. Many AAFS members are leaders in the development of these documents. As one of the largest and most diverse forensic science organizations in the world, it is appropriate that AAFS share its members' expertise to ensure that standards are set by the forensic science community itself. AAFS has completed its application to the American National Standards Institute (ANSI) to become an accredited SDO. A wholly owned subsidiary corporation entitled the AAFS Standards Board, LLC has been developed to provide the mechanics of the Academy's SDO activities. As an accredited SDO, AAFS will be able to coordinate the approval of proposed standards to become American National Standards (ANS).

Numerous industries, government agencies, and consumers outside of forensic science rely on voluntary consensus standards to direct them in their processes. The National Technology Transfer and Advancement Act (NTTAA) of 1995 and the Office of Management and Budget Circular A119 requires federal agencies to adopt private sector standards, particularly those developed by SDOs, wherever possible in lieu of creating proprietary, non-consensus standards. While there are many accredited SDOs supplying forensic-specific standards, the overall process of standard development is not always clearly understood. All ANSI-accredited SDOs must follow the "ANSI Essential Requirements: Due Process Requirement for American National Standards." These requirements focus on ensuring the standards development follow a procedure that is open to all interested parties, is balanced to allow parties equal participation, no individual or group can dominate the procedure, and that due process including mandatory public review and comments is allowed. All comments received concerning a proposed standard must be addressed prior to final vote of the standard. The Essential Requirements also demand that final consensus must be achieved before a standard is allowed to go forth.

The AAFS SDO process will be run by the Academy Standards Board (ASB). The ASB will conduct their work in a manner that is open to public scrutiny and provide every stakeholder an opportunity to be heard, without dominance by any party, in compliance with national and international standard development procedures. The ASB will appoint a Consensus Body technical committee for each proposed standard. The Consensus Bodies will be comprised of volunteers from relevant and interested parties both within the Academy and the forensic science community as a whole. These Consensus Bodies will be responsible for creating and approving consensus standards to be submitted to ANSI via the ASB for approval as an American National Standard. The ASB will conduct this standards development in accordance with the requirements of ANSI's Essential Requirements for balance, lack of dominance, due process, and consensus. The Consensus Bodies will consider all public comments, views and objections to ballots, and resolve all negative comments prior to approving the proposed standard. The ASB process will be open and balanced and will encourage public comment.

This presentation will provide an overview of how the standards development process works and detail the specifics of the Academy Standard Board's process including a specific outline of each step in the ASB standard development procedures. Issues such as how balance is achieved within a Consensus Body, how interested parties are defined, and resolution of public comments will be discussed. Information will also be provided on how AAFS members and the forensic science community can participate in the ASB process and the Consensus Bodies. Time for questions and discussion will allow participants to fully understand the standard development process and the Academy's commitment to ensuring the quality of the standards development.

Standards Development, SDO, ANSI

LUNCHEON SESSIONS

L1 Working Stiff: Forensic Training & Public Relations in a Digital Age

Judy Melinek, MD, PathologyExpert Inc, 3739 Balboa Street, #102, San Francisco, CA 94121; T.J. Mitchell, BA*, PathologyExpert Inc., 3739 Balboa Street, #102, San Francisco, CA 94121; and Lindsey C. Thomas, MD, Hennepin County ME, 530 Chicago Avenue, S, Minneapolis, MN 55415*

After attending this presentation, attendees will understand: (1) how to write clearly and effectively about forensic science; and, (2) how to use public relations and social media to respond to breaking news.

This presentation will impact the forensic science community by helping forensic professionals understand how to effectively communicate difficult forensic cases to a lay public.

Just two months before the September 11th terrorist attacks, Dr. Judy Melinek began her training as a New York City forensic pathologist. With her husband, T.J., and their toddler, Daniel, holding down the home front, Dr. Melinek threw herself into the fascinating world of death investigation — performing autopsies, investigating death scenes, and counseling grieving relatives. Working Stiff chronicles Dr. Melinek's two years of training, taking readers behind the police tape of some of the most harrowing deaths in the "Big Apple," including a firsthand account of the events of September 11th, the subsequent anthrax bio-terrorism attack, and the disastrous crash of American Airlines Flight 587.

Lively, action-packed, and loaded with mordant wit, Working Stiff offers a firsthand account of daily life into one of America's most arduous professions and the unexpected challenges of shuttling between the domains of the living and the dead. The body never lies — and through the murders, accidents, and suicides that land on her table, Dr. Melinek lays bare the truth behind the glamorized depictions of autopsy work on shows like CSI and Law & Order to reveal the secret story of the real morgue.

Dr. Melinek will discuss how she and her writer husband collaboratively turned her daily journal about her forensic fellowship training at the New York City Office of the Chief Medical Examiner into a New York Times bestselling book. Writing clearly and effectively about forensic science draws candidates to professional training programs, increases the credibility and public profile of forensic scientists, and has the potential to increase both local and federal funding. Dr. Melinek will emphasize that in the digital age, where Twitter®, Facebook®, Instagram™, TV news, and bloggers set the tone and control the narrative around breaking cases, forensic scientists cannot continue to hide from the press behind an autopsy table or lab bench. Offices need to develop a public relations profile and utilize media relations to respond independently to press and public inquiries consistently — not just when there is a scandal or a high-profile case.

Forensic Pathology, Public Relations, Training

L2 Operation Lima Sea — Unidentified Remains of a Human Torso in Queensland, Australia: Case Report on the Collaborative Investigative and Novel Anthropological (Forensic) Responses in the Establishment of Identification

Donna M. MacGregor, MSc, Queensland University of Technology, Skeletal Biology and Forensic Anthropology Res Lab, School of Biomedical Sciences, Faculty of Health,, Brisbane, Queensland 4001, AUSTRALIA; Mikaela S. Reynolds, MSc, Level 5 Q Block,, 2 George Street, Gardens Point, Brisbane, Queensland 4001, AUSTRALIA; and Jon E. Birt, BA*, Queensland Police Service, Homicide Investigation Unit, Police Headquarters, 200 Roma Street, Brisbane 4073, AUSTRALIA*

After attending this presentation, attendees will better understand: (1) the extensive contemporary investigative processes involved in the establishment of identification employed by the Queensland Police Service; and, (2) how the integration of novel anthropological and forensic processes assisted the investigation process.

This presentation will impact the forensic science community by demonstrating the tenacity of investigators from the Homicide Investigation Unit, Queensland Police Service to pursue all potential fields of inquiry to establish victim identification or victimology.

In October 2013 at a regional center in Southeast Queensland, Australia, Queensland Fire and Rescue (QFRS) were called to a grass fire. Once extinguished, QFRS located the remains of a human torso. The head and hands had been severed, and the lower body from the mid lumbar region had also been removed. The head, hands, and lower body have never been located. Due to the limited nature of the remains, standard confirmatory identification techniques of fingerprints and dental records could not be utilized in this matter. DNA was collected; however, it did not match any national database. Familial DNA was investigated; this too presented no matches. Toxicology was also conducted for a full drug screening and a number of prescription medications were identified. Investigators conducted exhaustive searches of mobile phone tower activity, Medicare files, immigration files, and interstate missing person searches in an attempt to identify the remains.

The investigators then engaged the services of their police anthropologist to assist in the identification process. Using Multi-Slice Computed Tomography (MSCT) Digital Imaging and Communications in Medicine (DICOM) data (0.5/0.3mm) of the torso collected during the standard pre-postmortem scanning procedure at the Brisbane Mortuary, 3D virtual reconstructions of the bone surfaces, also called isosurfaces, were created. The virtual isosurface models were uploaded into a specialized 3D software program, Geomagic® Design™ X, where virtual measurements were conducted to determine sex and stature. The measurements were conducted using a new and novel protocol developed by the Skeletal Biology and Forensic Anthropology Research Laboratory (SBFAR) at the Queensland University of Technology, Brisbane.1 The virtual measurements were collected from various bones within the torso including the humerii, scapula, and clavicle. An attempt was made to determine age of the individual using the sternal end of the fourth rib; however, the CT resolution and small surface area presented difficulties in age determination other than determining the individual was an adult. Subsequently, discussions between the anthropologist and investigators resulted in an application to the State Coroner of Queensland that was supported to have the sternal rib end of the fourth rib excised from the torso, then macerated (i.e., soft tissue removed from the bone) using dermestid beetles. A final age range, sex, and stature were provided to investigators.

Ultimately in July 2014, the prescription medication information collected from the toxicology report matched with the anthropological information obtained from the CT data and rib maceration and assisted in the identification. The contributions of the "virtual" anthropological input into this matter were a first for Queensland. The utility of CT data proved extremely useful in providing a timely anthropological profile to the investigation team and in reducing the need to macerate the entire torso as would be warranted by traditional anthropological techniques to develop an anthropological profile. This matter also exemplifies the importance of collaboration between the various agencies and specialists involved in homicide investigations to achieve a successful outcome.

Reference(s):

1. Reynolds, Mikaela S. (2014) Stature estimation of a contemporary Australian sub-population: an evaluation of the Trotter and Gleser method using computed tomography of the femur. Masters by Research thesis, Queensland University of Technology.

Unidentified Human Remains, Virtual Anthropology, Investigative Process

WORKSHOPS

W1 Information Does Exist Beyond the First Page of Your Google® Search! Tools and Strategies for Forensic Science Literature Searching and Use

John M. Butler, PhD, NIST, 100 Bureau Drive, MS 4701, Gaithersburg, MD 20899; Jeff Teitelbaum, MS*, 2203 Airport Way, S, Ste 250, Seattle, WA 98134; Susan Makar, MA*, NIST, 100 Bureau Drive, MS 2500, Gaithersburg, MD 20899; Amanda Malanowski, BS*, NIST, 100 Bureau Drive, MS 2500, Gaithersburg, MD 20899; Melissa K. Taylor, BA*, 100 Bureau Drive, Gaithersburg, MD 20899; and Matthew R. Wood, MS, Ocean County Sheriff's Dept, Forensic Science Laboratory, Toms River, NJ 08753*

After attending this presentation, attendees will understand the value of forensic science literature and how to search and use the literature to research topics of work-related interest, such as developing appropriate training materials and preparing for admissibility hearings.

This presentation will impact the forensic science community by serving as a venue for understanding the importance and value of forensic science literature and tools for finding information of interest to practitioners, researchers, and students.

Scientific fields are benefited by having access to and active use of published literature. Researchers publish their work to share knowledge with others and to gain recognition and prestige for their efforts. Knowing where to look in the scientific literature can help find answers to specific questions faced by forensic scientists. This workshop will present current practices and tools for discovering, using, and analyzing the literature from various forensic science disciplines. Strategies for reading, writing, and storing information on scientific publications will also be discussed.

As Jeff Teitelbaum notes: "Because there is no central repository for forensic science information, and because of the sheer number of disciplines under the forensic science umbrella, forensic scientists are often unable to locate material that is relevant to their needs."[1] The ability to use carefully selected keywords and keyword combinations to yield valuable information from publicly accessible search engines and databases such as Google®, PubMed®, Google® Scholar™, Google® Books™, WorldCat®, and the National Criminal Justice Reference Service will be demonstrated. Researchers having access to Web of Science™ or other commercial search tools and databases can extend their information searches.

Example searches will be conducted using both free resources available to any practitioner and specialized literature databases available to academic researchers and students. These case examples from multiple forensic disciplines will illustrate the challenges of searching the forensic science literature. Experienced reference librarians from the Washington State Patrol Crime Laboratory and the National Institute of Standards and Technology will demonstrate the types of searches that can be performed to provide information in addressing specific questions that may arise in training, troubleshooting, or testimony on the admissibility of a technique as well as to assess the impact of forensic science publications and collaborations. Approaches to visualize connections in the literature and to reflect the impact of specific authors or articles will also be discussed.

This workshop will include information on current journals by forensic discipline and a listing of literature-searching websites and other resources. The information being shared in this workshop is particularly timely given the January 2015 views document from the National Commission on Forensic Science regarding criteria for foundational, scientific literature supportive of forensic practice. Updates on ongoing activities involving analysis of forensic literature by the American Association for the Advancement of Science will be provided. Finally, a vision of potential future information resources to address current limitations of accessibility of forensic science literature will be shared.

Reference(s):

1. Teitelbaum J. An improved forensic science information search. *Forensic Science Review* 27(1):41–52; 2015.

Forensic Literature, Search Strategies, Information Resources

W2 Advanced Mass Spectrometry (MS) Techniques for Forensic Analysis: What Does the Future Hold?

Sherri L. Kacinko, PhD, 3701 Welsh Road, Willow Grove, PA 19090; Kenyon M. Evans-Nguyen, PhD*, 401 W Kennedy Boulevard, Tampa, FL 33606; David M. Schwope, PhD*, Aegis Sciences Corporation, 365 Great Circle Road, Nashville, TN 37228; Adam B. Hall, PhD*, Northeastern University, 360 Huntington Avenue, 140 The Fenway, 421TF, Boston, MA 02115; Jillian K. Yeakel, MS*, 3864 Courtney Street, Ste 150, Bethlehem, PA 18017; and Jason E. Schaff, PhD*, 2501 Investigation Parkway, Rm 4220, Quantico, VA 22135*

After attending this presentation, attendees will be better able to evaluate and select advanced MS techniques for solving various analytical problems in forensic science, including identification of unknowns, rapid throughput approaches to forensic sample preparation, novel ionization, and fragmentation approaches in hyphenated mass spectrometric techniques.

This presentation will impact the forensic science community by introducing attendees to some of the most recent advances in MS technology and their potential application to solve challenges in forensic investigations. This workshop has a strong interdisciplinary focus.

Advances in MS technology over the past decade profoundly affect the way forensic toxicologists and drug chemists approach screening of samples for the presence of controlled substances and other drugs. Immunoassay and Gas Chromatography/Mass Spectrometry (GC/MS) analysis, once considered the "gold standard" for initial detection and exclusion of specific drugs/drug classes, are being replaced by Liquid Chromatography/Mass Spectrometry (LC/MS) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) methods due to increased availability of new instrumentation and performance improvements in existing technologies. An overview of the application of MS to drug screening in a modern toxicology laboratory, illustrated with casework data, will be provided. Topics will include the reasons behind the trend toward LC/MS analysis, comparison of the advantages of different MS technologies, different models for sample screening, and the use and limitations of high resolution and accurate mass data.

Multiplexing is a high-throughput solution which allows minimization of MS idle time by using two High-Performance Liquid Chromatography (HPLC) systems configured to one detector. This technique allows overlap of chromatographic runs while collecting spectrometric data in predefined windows. Two HPLC systems also permit simultaneous analysis by two methods employing different mobile phase systems. A 2D LC/MS/MS system increases sample throughput while preserving equilibration time.

Stable Isotope Ratio Mass Spectroscopy (IRMS) has been used in geochemistry and other fields for decades. Since the introduction of Gas Chromatography/Combustion IRMS (GC/C/IRMS), Compound-Specific Isotope Analysis (CSIA) has become increasingly utilized to determine isotopic composition in various fields. Several forensic applications of CSIA have been documented, including: determination of illicit drug preparations, identification of counterfeit pharmaceuticals, determination of doping in sports, and investigation of ignitable liquids and explosives; however, GC/C/IRMS is not without challenges. Labor-intense sample preparation, poor sensitivity, delicate instrumentation, and lack of uniform standards are difficulties which have hindered widespread adoption. This presentation will introduce attendees to GC/C/IRMS and detail current forensic GC/C/IRMS applications, with a focus on sports-doping steroid analysis. Present state-of-the-art and future possibilities of the methodology will be presented.

Several technologies for rapid and/or on-site MS have migrated from prototype instruments in research laboratories to fully validated commercial systems. Unreliability of color tests for preliminary identification of emerging synthetic drugs have increased interest in this instrumentation in forensic science. Additionally, fieldable MS has been used extensively in battlefield forensics for on-site identification of drugs and explosives. Currently, fieldable mass spectrometers can be used for preliminary identification and in the near future, these instruments could be used for rapid definitive identification in the field. Coupling simplified sampling strategies such as ambient ionization, solid phase microextraction, and thermal desorption have been key to success with these instruments. An overview of currently available instrumentation, primarily GC/MS and ion-traps, and their implementation by forensic scientists will be presented as well as a discussion of emerging instrumentation.

In the final presentation, attendees will be left with some thought-provoking ideas of where the discipline of forensic MS is headed in the future. From the days of Gas Chromatography/Flame Ionization Detector (GC/FID) use as a confirmatory approach to the current uses of LC/MS/MS and higher resolution options, the forensic science community has adapted to changes in the analytical technologies and implemented them to address challenges in casework. Technology will continue to advance and provide new opportunities for addressing challenges in our everyday work. This presentation will outline anticipated changes that will occur in the forensic drug and toxicology communities over the coming years.

Mass Spectrometry, Sample Preparation, Advanced Techniques

W3 How and Why You Can and Should Integrate Advanced Imaging Techniques Into Your Daily Autopsy Practice

Keith Pinckard, MD, PhD, Travis County Medical Examiner (Austin), 1213 Sabine Street, Austin, TX 78701; Evan Matshes, MD*, Academic Forensic Pathology Inc., 6927-48th Street, SE, Ste 200, Calgary, AB T2C5A4, CANADA; Sam W. Andrews, MD*, Travis County Medical Examiner's Office, 1213 Sabine Street, Austin, TX 78701; and Vivian Snyder, DO*, 572 Sheridan Square, Apt 3, Evanston, IL 60202*

After attending this presentation, attendees will: (1) understand the concept of "disciplinary cross-over" within medicine; (2) understand the "toolbox" approach to modern forensic pathology; (3) review the core science behind plain film radiography, Postmortem Computed Tomography (PMCT), and Postmortem Magnetic Resonance (PMMR); (4) understand practical applications of imaging techniques as they apply to natural and non-natural deaths; (5) understand the concept of "Targeted Tissue Assessments" (TTA) in the context of whole body PMCT and why TTA is not a partial autopsy; and, (6) review administrative considerations as they pertain to the installation and routine utilization of advanced imaging techniques.

This presentation will impact the forensic science community by exploring how forensic pathologists can and should take ownership of advanced imaging techniques in their daily practices.

The tools available to forensic pathologists have undergone few significant upgrades over the past few decades. While initially considered a luxury to many, plain film radiography has become standard technology in autopsy suites — so much so that National Association of Medical Examiners (NAME) accreditation of any system of death investigation requires access to a simple "X-ray" machine. Despite routinely ordering and interpreting radiographs, no forensic pathologist would describe himself/herself as a radiologist. Rather, like clinicians, they are making use of radiographs in their own practices — an example of "disciplinary cross-over" within medicine.

Although clinical medicine has quickly adopted more modern and advanced technologies, such as computed tomography and magnetic resonance imaging, forensic pathologists have been reticent to embrace such tools. Reasons for this include the high costs of installation and operation, training requirements for forensic pathologists and their support staff, and generalized "apprehension" about the adoption of new technologies. Some forensic pathologists resist the use of advanced radiologic techniques in their practices, citing concerns that "radiologists will take away their jobs." This concern ignores the reality that there is a paucity of radiologists interested in postmortem work, particularly in the context of the vast differential in remuneration between clinical radiology and forensic pathology. Furthermore, even if there were a plethora of radiologists interested in forensic imaging, none of them have the statutory authority or duty to investigate and certify death.

A handful of mortuaries have adopted Postmortem Computed Tomography (PMCT), and a very small number have adopted Postmortem Magnetic Resonance (PMMR) scanning. Some institutions make use of these technologies to augment their daily practices; others have taken a more focused "research-based" approach. This workshop takes a practical approach to the introduction and utilization of advanced radiologic techniques into daily forensic pathology practice. While extremely useful, PMCT is not an across-the-board replacement for autopsies; however, it is an excellent substitute for internal examination across broad categories of commonly investigated deaths. In general, PMCT can be regarded as either supplementing traditional examinations or supplanting them. Categories of death that traditionally undergo invasive autopsy, but that now can most often undergo only external examination with PMCT include: accidental blunt trauma, suspected trauma in the elderly and other at-risk adults, suicidal violence, some types of sudden natural death, and certain types of mechanical asphyxia, including choking on food.

Although rarely used, PMMR does have a solid and important function within the forensic pathology setting; the roles and limitations of this important, but very expensive technology will also be reviewed.

After this presentation, attendees should have grounded expectations for the important strengths and weaknesses of both PMCT and PMMR and should also understand fundamental administrative considerations regarding adoption, installation, and utilization of new technologies within systems of death investigation. Without any doubt, the introduction of advanced radiologic techniques into the autopsy suite represents the most important advancement in forensic pathology "tools" in the past century. This session will help to convince attendees that advanced imaging is within reach and can and should be utilized whenever possible within daily practice.

Postmortem Imaging, Autopsy, PMCT

W4 A Cloud Descends on the Courtroom: The Impact of Cloud Computing on Evidence in the Courtroom

Mark Pollitt, PhD, Digital Evidence Professional Services, Inc, 8509 Nicole Court, Ellicott City, MD 21043; Christopher J. Plourd, JD*, Superior Court, 939 Main Street, El Centro, CA 92243; Mary F. Horvath, MFS*, 6786 N Stuart Road, King George, VA 22485; Josiah Dykstra, PhD*, 1739 Carriage Lamp Court, Severn, MD 21144; Henry R. Reeve, JD*, Denver District Attorney's Office, 201 W Colfax Avenue, Ste 801, Denver, CO 80202; Abagail Abraham, JD*, AOL, 22000 AOL Way, Dulles, VA 20166; and Andrew Neal, MS*, TransPerfect Legal Solutions, 1717 Main Street, Ste 4450, Dallas, TX 75201*

After attending this presentation, attendees will better understand how the rapidly expanding technologies surrounding the storage and distribution of information and applications using what is commonly called "cloud computing" are impacting investigators, forensic examiners, and lawyers from the crime scene to the courtroom.

This presentation will impact the forensic science community by providing a brief tutorial on these technologies, giving attendees an appreciation of the difficulties in acquiring, analyzing, introducing, authenticating, and evaluating information stored "in the cloud." After attending this presentation, participants will be able to evaluate how these technologies are changing the practice of both law and forensic science.

The National Institute of Standards and Technology defines cloud computing as: "…a model for enabling ubiquitous, convenient, on-demand network access to a shared pool of configurable computing resources…"[1] Government and industry have rapidly adopted the use of massive computer resources to provide information storage and applications online for both internal and external use. While some of the uses of cloud computing are fairly obvious, such as web-based email, social media, and electronic commerce, the cloud technologies are increasingly being used for third-party applications and even in-house computing systems for handling evidence. One source suggests that by next year, more than one-third of all personal data in the world will be stored in the cloud.[2] This massive amount of data, coupled with the location of the computing resources distributed across the globe, presents a rapidly evolving set of problems for investigators, information security professionals, forensic scientists, and the legal community. Investigators and digital forensic examiners are already facing difficulties in locating, collecting, and utilizing cloud-based storage and applications. The courts are beginning to face challenges to admissibility and determining the reliability of proffered evidence.[3] For the entire forensic community, there are concerns regarding the privacy, confidentiality, and integrity of cloud-based data and applications.[4] Clearly, the impact of the movement of data and applications is beginning to have a major impact on the practice of forensic science.

This presentation will bring together computer scientists, forensic practitioners, information security practitioners, lawyers, and judges to discuss many of the emerging issues in this rapidly evolving field. Topics include: What is the Cloud, Legal and Practical Issues in Evidence Collection, Foundation and Admissibility of Cloud-Based Evidence, and Security and Privacy in the Cloud.

Reference(s):

1. National Institute of Standards and Technology. *The NIST Definition of Cloud Computing*. Special Publication 800-145. 2011.
2. Butler B. Gartner: 1/3 of consumer data will be stored in the cloud by '16. *Network World*. 2012.
3. Wilson D. Legal Issues with Cloud Forensics. *Digital Forensic Investigator News*. 2015.
4. National Institute of Standards and Technology. *Guidelines on Security and Privacy in Public Cloud Computing*. 2011.

Cloud Forensics, Digital Evidence, Computer Evidence

W5 UVIS Dental Identification Module (UDIM) — A Hands-On Workshop

Kenneth W. Aschheim, DDS, 44 E 67th Street, New York, NY 10065; Lawrence A. Dobrin, DMD*, New York City OCME, 471 E Westfield Avenue, Roselle Park, NJ 07204; Naeem Ullah, BS*, 421 E 26th Street, 10 Fl, Rm 1019, New York, NY 10016; Frank DePaolo, BS*, New York City OCME, 520 First Avenue, Rm 123, New York, NY 10016; John Fudenberg, MBA*, 1704 Pinto Lane, Las Vegas, NV 89106; Edward E. Herschaft, DDS*, UNLV School of Dental Medicine, 1001 Shadow Lane, MS 7412, Las Vegas, NV 89106-4124; John P. Demas, DDS*, 8814 Fort Hamilton Parkway, Brooklyn, NY 11209; and Davin Faulkner, DMD*, 290 Maddelena Avenue, Las Vegas, NV 89183*

After attending this presentation, attendees will: (1) become familiar with the functionality and features of the Unified Victim Identification System (UVIS), the UVIS Case Management System (UVIS-CMS), and the UDIM Stand Alone (UDIM-SA) software systems; and, (2) gain experience in order to enter, search, and compare antemortem and postmortem dental data utilizing the UDIM-SA software to identify a decedent.

This presentation will impact the forensic science community by providing attendees with a working knowledge of the UDIM, a component of a complete forensic case management system.

Following the World Trade Center and American Airlines Flight 587 disasters, the Office of Chief Medical Examiner of the City of New York (OCME) undertook a multi-year project of creating a browser-based fatality management system, UVIS, to aid in the identification of mass fatality victims. Based on research and lessons learned following these Multiple Fatality Incidents (MFIs), a new dental module, UDIM, was introduced to the forensic odontology community. The goal of this workshop is to provide hands-on experience with this module.

UVIS is a comprehensive disaster-management software system designed to coordinate all of the activities related to missing persons reporting and victim identification. By integrating key functions in Disaster Victim Identification (DVI), the software coordinates all the essential tasks necessary to develop an accurate manifest of potential victims as well as coordinating all components of remains management.

The UDIM can function as either an integrated module within UVIS or as a stand-alone dental identification software program, UDIM-SA. It is designed to be used for both daily operations or as an MFI dental identification module.

UDIM-SA is capable of recording detailed charting with its "click-to-code" interface, conduct complex searches utilizing optimized state-of-the-art unified ranking algorithms, and has the ability to find and highlight anomalies. The state-of-the-art coding interface not only allows the coding of restored surfaces, but also more complex restorations such as root canals, posts, implants, and implant abutments. Another unique feature of UDIM-SA is its detailed "self-correcting" coding interface. Built-in reference tables prevent the forensic odontologist from entering illogical or contradictory codes. UDIM also uses a unique color-coded comparison odontogram to decrease reconciliation times. This color coding allows for a more simplified comparison process of antemortem and postmortem data by highlighting explainable and unexplainable discrepancies. In addition, UDIM has extensive partial jaw fragment management, linking and joining of specimens, and unlimited image importation. UDIM-SA also has the ability to integrate with digital radiographic software which, with the unlimited image importation, makes UDIM a fully paperless dental forensic management system.

The current version of UDIM has been enhanced with a more robust security system. With four different odontology roles, administrators can control access to numerous submodules. From the forensic dental operator with read-only access to a full administrator, UDIM-SA allows a municipality to customize the software access based on the odontologist's skill and experience. The current version of UDIM has been upgraded to allow for the exportation of data and a customizable translation table allows conversion to other coding systems including the new Type-12 Dental Data set for the American National Standards Institute/ National Institute of Standards and Technology-Information Technology Laboratory (ANSI/NIST-ITL).

Participants will receive in-depth didactic instruction regarding the use of the program. This will be followed by hands-on training utilizing the UDIM-SA program to input both antemortem and postmortem dental information and perform reconciliation of this data utilizing UDIM advance comparison features.

UDIM, Dental Identification, Forensic Odontology

W6 Frequency Occurrence in Handwriting and Hand Printing Characteristics

Thomas W. Vastrick, BS, 522 S Hunt Club Boulevard, Ste 217, Apopka, FL 32703; Ellen M. Schuetzner, BA*, 6348 N Milwaukee Avenue, #161, Chicago, IL 60646-3728; and Mark E. Johnson, PhD*, UCF - Statistics Dept, University of Central Florida, Dept of Statistics, Orlando, FL 32816*

After attending this presentation, attendees will have a significant understanding and appreciation of the statistical bases for handwriting comparisons and how to present such information in court.

This presentation will impact the forensic science community by providing information that can be used in court cases in which statistical foundation and probability become weight or admissibility issues.

Handwriting comparison has a rich history in both documented methodologies and admissibility in courts across the United States. During a literature search, numerous small and moderate studies were found that collectively provided significant, but not necessarily proper, foundation for questions of uniqueness and probability of handwriting and hand printing characteristics. In 2009, the National Research Council Report, Strengthening Forensic Science in the United States: A Path Forward, sought to make recommendations to strengthen forensic disciplines through strengthening the statistical foundations for each discipline. In response to that report, workshop presenters Vastrick and Schuetzner designed and instituted a four-year comprehensive study of frequency occurrence in handwriting and hand printing characteristics within the United States. Vastrick and Schuetzner were joined by Mark Johnson and Michele Boulanger, both statisticians with experience in forensic science and standards of methodologies. Johnson and Boulanger were tasked with being the driving force behind the project and developing the procedural methodologies using Vastrick and Schuetzner as subject matter experts. The purpose was to make this a statistics project about handwriting comparison as opposed to a handwriting project with statistics. The project was funded by the National Institute of Justice (NIJ).

Based on Handwriting Identification: Facts and Fundamentals, along with current population statistics, Johnson and Boulanger produced a stratified sampling frame that best represented population sampling.[1] In addition, factors potentially influencing handwriting such as age, handedness, and education were taken into consideration in developing the frame. One aspect of this workshop will be to understand the population sampling selection process and attendees will have opportunities for some hands-on experience with this subject. Within the final report are the eventual results of the population sampling and these results will be highlighted to workshop attendees. It is hoped that this information will provide the basis from which future sample collections are made for handwriting collection purposes. In addition, this project provided quantitative results to the lists of factors that affect handwriting and these results will be discussed.

Each phase of the project had to undergo pilot testing and attendees will receive experience in each of the processes. The most influential pilot test was the Attribute Agreement Analysis (AAA). The statistical aspects of the AAA approach are embodied in the International Organization for Standardization Technical Report (ISO TR) 14468. Workshop attendees will conduct an experimental AAA.

Attendees will receive detailed instruction into the process of selecting and testing handwriting characteristics for this project. Originally, there were more than 2,500 characteristics selected, but pilot testing reduced this number significantly to 786. Attendees will learn about the initial selection process and the testing processes that were used.

Attendees will receive background information into the data entry process and will have the opportunity to use a database in order to understand the methods used in this research.

Product rule analysis was conducted on sets of specimens resulting in 97.01% of all cursive and 98.96% of all hand-printed feature pairs having a correlation of plus or minus 0.2 for which the product rule is satisfactory. Attendees will have the opportunity to use both the hand printing and cursive data spreadsheets to manually test the independence or interdependence of several pairs of characteristics.

Confidence limits are the range in which population samplings can be statistically accepted as being within 95% confidence level. This range will be explained and the mathematical equation used to establish 95% confidence limits will be applied several times in planned exercises in order to provide experience and understanding of this factor to the workshop attendees. The mathematical equation is moderately complex, so several exercises to establish a level of comfort will be required.

Each attendee will be provided a mock query and given several test samples from which they will have the opportunity to enter the data and receive a canned report as to the frequency occurrence of each character entered, their corresponding 95% confidence limits, and the results of applying the product rule to their results.

Each attendee will receive an electronic packet containing the final report of the research project, copies of historical papers concerning statistics in handwriting, copies of charts reflecting features that affect handwriting, publications concerning population statistics within the United States, a blank database, the final project spreadsheet of results, and a mock query. Also included will be several exercises as previously described.

Attendees will be cautioned about potential abuse and misuse of the project results and cautioned regarding the measures taken to limit the potential for abuse. Attendees will use the query in some of these ways to illustrate the potential for misuse.

The workshop will close with a roundtable discussion as to uses for this information and future enhancements to the

project. Attendees will be encouraged to solicit other forensic document examiners to receive training in the use of the material from this workshop. Due to its overall complexity, it would not be desirable to self-train.

Reference(s):

1. Huber R.A., Headrick A.M. *Handwriting Identification: Facts and Fundamentals*. Boca Raton, FL: CRC Press, c1999.

Statistics, Frequency Occurrence, Handwriting

W7 Extreme Violence — Military vs. Civilian Crime Scene Investigation (CSI) Cases — Forensic Analysis and Disciplines in Practice

Brian L. Janysek, MFS, 2521 Hunter Mill Road, Oakton, VA 22124; Ryan P. Brokaw, MFS*, U.S. Army CID, Fort Benning CID Office, 7235 Gillespie Street, Bldg 108, Fort Benning, GA 31905; Scott Roeske, MFS*, 2635 Miner Road, Fort Sill, OK 73503; Jessica Ann Veltri, MS*, U.S. Army CID, 22nd Military Police Battalion (CID), Bldg 3148 2nd Division Drive, Joint Base Lewis-McChord, WA 98433; Donald Hayden, MFS*, 292 Harbour Lane, Richmond Hill, GA 31324; Steven Geniuk, MS*, 108 S Johnson Street, Bldg 31022, Fort Huachuca, AZ 85613; and Curtis E. Sparling, MA*, 10077A Horizon Street, Joint Base Lewis-McChord, WA 98433-9567*

After attending this presentation, attendees will understand multiple crime scene investigation methods, including injury pattern analysis, post-blast analysis, mass murder crime scene processing, and methods for solving an array of violent crimes.

This presentation will impact the forensic science community by identifying detailed and relevant aspects into the dynamics of multiple violent crime scenes observed around the world. The crimes were investigated by the Military Criminal Investigative Organizations. The investigations will present multimodal approaches elaborating on crime scene processing, evidence collection, interrogation methodology, post-blast reconstruction, medicolegal death determinations, and judicial hurdles and findings.

A group of military Forensic Science Officers (FSOs), each bringing decades of federal law enforcement experience within the United States Army Criminal Investigation Division, will present four high-profile investigations from the last ten years as well as discuss detailed steps every CSI responder experiences to process death scenes. There will be an in-depth focus on the significant challenges faced during the investigations, including special crime scene issues, language and cultural barriers, the combat environment, and examination of a stale murder scene. This presentation will explore the interaction of multiple forensic disciplines — crime scene examination, skeletal recovery, forensic anthropology, forensic odontology, DNA and computer forensics — as well as the utilization of young forensic scientists who used one case to springboard their careers.

In March 2012, a United States Army infantryman left his small outpost in southeastern Afghanistan, undetected and without authorization, and began a horrific killing spree. When finished, he invaded five homes in two villages, murdered 16 Afghani civilians (mostly women and children), and seriously wounded 6 others. The subsequent outrage from the Afghanistan nation would prevent Army investigators from reaching the crime scenes for more than three weeks. How DNA, firearms, and tool mark evidence helped link the subject to his victims and the scene of his crimes will be discussed.

A then-26-year-old female went missing in Missouri in 1985. The civilian investigation went cold until mid-2005 when information as to the identity of the murderer was received by the local sheriff's office. The investigation developed the probable location of the remains and ground/air searches, combined with additional interviews, identified a pond as a probable burial site. In 2006, forensic science and forensic anthropology college students, supervised by their professors, were engaged in the recovery. After federal and state coordination, an excavation was accomplished, which resulted in the recovery of skull fragments and a full mandible. Positive identification was made through dental comparison and DNA analysis and, in 2008, the suspect plead guilty to first-degree murder and sentenced to life in prison.

Military law enforcement aspects in processing crime scenes will be presented. Topics include: jurisdictional concerns at military bases inside and outside of the United States; cooperation with local law enforcement; working with a limited suspect pool; scene response while deployed and in austere environments; an overview of scene response and processing techniques; interviews; and, the military justice system.

The next case is a detailed account of a suicide bomber in Afghanistan who killed 11 and injured 22 in 2011. The topics discussed will include investigative operations in Afghanistan during the height of the 2011 Allied surge, post-blast analysis, methods of clearing and collecting evidence from the scene with live ordinance and biohazards, projectile trajectory, pattern injury analysis, and the search for additional suicide bombers.

The final case details how a self-employed Information Technology (IT) computer repairman found digital evidence on the cloud that his wife was having an extramarital affair while she was deployed. The couple's daughters made posters welcoming the mother home, but failed to show up at the welcome home ceremony. The day she returned from deployment, her husband printed the emails, leaving them on the printer, then killed his oldest daughter while she slept. When the other daughter awoke, he killed her before committing suicide. Elements of the crime scene, the physical evidence, and the significant digital evidence on the cloud, which was later recovered and corroborated the affair, will be discussed.

Investigation, Violence, Multidisciplinary

W8 From the Ashes — Transforming the Response to Mass Disasters

Anjali A. Ranadive, JD, SciLawForensics, Ltd, 1834 Overlook Ridge Road, Brookings, SD 57006; Edward Mazuchowski II, MD, PhD*, 115 Purple Heart Drive, Dover AFB, DE 19902; Robert E. Barsley, DDS, JD*, LSU School of Dentistry, Oral Health Resources, Rm 5345, 1100 Florida Avenue, New Orleans, LA 70119; Noelle J. Umback, PhD*, OCME, Dept of Forensic Biology, 421 E 26 Street, New York, NY 10016; Dean M. Gialamas, MS*, Los Angeles County Sheriff's Department, Technology & Support Division, 12440 E Imperial Highway, Ste 650, Norwalk, CA 90650; Joanna L. Collins, MFS, 20079 Stone Oak Parkway, Ste 1105-215, San Antonio, TX 78258; and Mary B. Collins-Morton, MS*, FBI Academy, NCAVC BAU4, Quantico, VA 22135*

After attending this presentation, attendees will understand how different agencies integrate various areas of forensic science in mass fatality incidents and how various forensic service providers can collaborate within and with these agencies to prepare for mass disaster events. In addition, attendees will better understand how emerging technologies are being leveraged.

This presentation will impact the forensic science community by highlighting what has been learned from numerous natural and unnatural disasters and how the forensic science community working together can prepare to respond to future incidents by teaching how to process and investigate mass disaster scenes and evidence.

A mass fatality incident occurs when the local resources that are normally used in response to a fatality event are overwhelmed or have the potential to be overwhelmed. This varies widely depending on the size of the departments and the type of incident. In order to effectively manage these incidents and conduct appropriate medicolegal death investigations, it is necessary to have an integrated approach between various forensic science disciplines.

This presentation will provide an overview of how various areas of forensic science are integrated in mass fatality incidents.

The goal is to introduce forensic scientists working their first mass fatality scene to scientific and administrative procedures generally used in processing the scene. In many incidents, it is not known at the onset whether the incident was accidental or criminal in nature, so the scene must be treated in the same detailed manner in all cases until conclusions can be made. A number of cases that have caused these agencies to improve their procedures will be discussed. There is more coordination between all state, local, and federal law enforcement agencies than ever before, addressing, for example, preparation, jurisdiction, responsibilities, and task assignments.

At the scene, it is necessary to identify evidence, decontaminate the remains as necessary, systematically document and recover the remains and personal effects, provide transport and/or temporary storage of the remains, and generate and maintain a chain of custody of all evidence, remains, and personal effects. This presentation will provide an overview of these procedures and how they may change based on circumstances.

Various resources available to law enforcement and medicolegal agencies developing their continuity of operations, contingency plans, and other plans will also be discussed.

At the intake area of the autopsy operations site, all remains are triaged, photographed by a forensic photographer, and given a unique identifier. Remains are scientifically identified by integrating the skills of fingerprint specialists, forensic odontologists, and DNA specialists. Radiographs are performed by radiology technicians and forensic radiologists interpret the images. Examination of the remains is conducted by forensic pathologists, toxicology samples are taken, and all evidence is collected and released to the investigative agency. Forensic anthropologists assist in the triage and provide expert consultation. The forensic pathologist usually completes the death certificate and certifies the identification and cause and manner of death. The remains and personal effects are released to mortuary affairs and communication is made with the families.

After intake, the triaged and numbered remains are viewed by the dental team. Forensic odontology provides a highly accurate, rapid method of identifying remains and associating partial remains. Successful dental identification requires trained forensic odontologists and support staff, a means of locating and obtaining the antemortem dental information, sufficient space and support to perform the postmortem examinations, and adequate Information Technology (IT) support and resources. The dental team works closely with the other forensic disciplines and with the members of the family assistance group, which gathers antemortem information. The dental team makes identification recommendations on the ultimate identity and release of the remains.

Once the examinations in the morgue are finished, the DNA laboratory begins work. Samples received may be much different than standard postmortem samples in source, amount, and condition, depending on the incident. DNA may be the only method to identify partial remains, but it is crucial to collect from all samples regardless of other identification modalities, since reunification of as many remains as possible is a major goal. Laboratories may find themselves inundated with samples almost immediately; this includes remains, known samples, and other family samples. Having the correct data in an organized fashion facilitates identifications; therefore, DNA-relevant information must be collected by the Victim Information Center. Tremendous improvements have been made in DNA database programs (capacity for Short Tandem Repeat (STR), Y-chromosomal Short Tandem Repeat (Y-STR), and mitochondrial DNA data as well as metadata), which can be used as a stand-alone system for mass disasters. Since 9/11, methods for bone processing and developing mini-STRs for degraded samples have advanced greatly.

Preparing for a future yet-unknown mass disaster can assist when disaster does strike. Developing a program for preparedness and training across agencies can be daunting but well worth the effort. This presentation will describe developing a

model program to coordinate Orange County forensic operations stakeholders with a first-hand look at the process development, planning, and implementation of a simulated mass disaster; this will assist in developing programs in the participants' own jurisdictions.

Mass Disaster, Coordinated Response, Forensic Scientists

W9 Strategies for Scientific Problem-Solving With Physical Evidence

Rebecca E. Bucht, PhD, Pietarinkatu 11 A 13, Helsinki 00140, FINLAND; Patrick Buzzini, PhD, Sam Houston State University, Chemistry/Forensic Science Bldg, 1003 Bowers Boulevard, Box 2525, Huntsville, TX 77314; Peter R. De Forest, DCrim, Forensic Consultants, PO Box 141, Ardsley, NY 10502; Douglas M. Lucas, DSc*, 5280 Lakeshore Road, #1111, Burlington, ON L7L 5R1, CANADA; Pierre A. J-L. Margot, PhD*, University of Lausanne, Ecole de Sciences Criminelles, Batochime, Lausanne 1015, SWITZERLAND; Alastair Ross*, Australia New Zealand Policing Advisory Agency, Level 6, Tower 3, World Trade Center, 637 Flinders Street, Melbourne 3005, AUSTRALIA; and Sheila Willis, PhD*, Forensic Science Ireland, Garda HQ, Phoenix Park, Dublin, IRELAND*

After attending this presentation, attendees will have better knowledge of and more insight into the development, underpinnings, and potential value of criminalistics from the perspective of experienced and knowledgeable forensic scientists.

This presentation will impact the forensic science community by addressing the often-overlooked but crucial question of which examinations to perform for a given case, how that decision-making is currently organized, and how it might be improved.

With the rapid growth of the forensic science industry, focus has been more on what scientific techniques to apply to questions of the law and how to increase the number of analyses done than on how those methods are applied. "Progress has been technical rather than fundamental, practical rather than theoretical, transient rather than permanent."[1] This quote in Paul Kirk's article, *The Ontogeny of Criminalistics,* still rings true.

Forensic science has developed on many fronts and there has been an increase in the demand for forensic science services, both in the number and size of forensic laboratories and in the number of university degree programs in forensic science. Efforts in the domain of laboratory accreditation, proficiency testing, and expert certification have contributed to ensuring the quality of the analytical work being performed. These, along with impressive technological advances, are obviously perceived as positive developments; however, there have also been more negative trends: forensic scientists are increasingly confined to the role of reactive technicians and rarely address the complete physical evidence investigation, especially in the context of complex and non-routine cases, and requests submitted to the forensic laboratory are often limited to factual, technical reports rather than the more complex and often more useful evaluative reports and reconstructions.

Responsibility for defining the scientific problem to be solved on the front end and of interpreting the significance of the scientific results within the context of the case on the back end lie primarily with non-scientist personnel. The research being conducted in the forensic science field also rarely addresses this front end and back end decision making. As a consequence, the contribution of forensic science rarely reaches its full potential.

The presenters bring with them a wealth of knowledge and experience concerning the evolution of forensic science industry in several countries. Along with a summary of how forensic science has evolved in their own jurisdictions, they will present their views on the key elements required for the optimization of the contribution of forensic science to criminal justice questions, particularly with regard to complex and non-routine cases, volume crime, and providing guidance to criminal justice policymakers.

Practical, interactive exercises will also be included to illustrate the key points made by the presentations.

Reference(s):

1. Kirk P.L. The Ontogeny of Criminalistics, 54 J. Crim. L. and Criminology 235 (1963).

Case Assessment, Forensic Intelligence, Complex Cases

W10 Practical Homicide Investigation®: An Evaluation of Homicides Involving Child Victims, Child Offenders, and Equivocal Death Investigations

Vernon J. Geberth, MS, MPS, Practical Homicide Investigation, PO Box 105, Marco Island, FL 34146; Barbara C. Wolf, MD*, District 5 MEO, 809 Pine Street, Leesburg, FL 34748; Thomas C. McAndrew, MA*, Pennsylvania State Police, 5933 Derick Drive, Orefield, PA 18069; and Andrea Zaferes, BA*, PO Box 601, Shokan, NY 12481*

After attending this presentation, attendees will better understand the unique aspects of child homicides and the dynamics involved in cases in which children are offenders. In addition, the presenters will discuss equivocal deaths and aquatic death investigations as well as the application of professional homicide investigation and medicolegal analysis to these events.

This presentation will impact the forensic science community by providing and familiarizing forensic scientists and investigators with the art and science involved in the professional investigation and medicolegal analysis in homicide investigations specifically as they relate to child homicides and equivocal death inquiries.

The investigation, medicolegal evaluation, and prosecution of child abuse-related fatalities are different from other types of homicides. The death scenes are often equivocal and open to different interpretations as to the cause and manner of death, sometimes due to manipulation of the scene by the offender. Likewise, the autopsy findings may be non-diagnostic and even absent, the so-called "negative autopsy." Such deaths may present as homicides, suicides, accidents, or natural deaths and may be altered by the offender to misdirect the investigation through staging of the scene and/or posing or mutilation of the body. They are open to interpretation pending further information of the facts, the victimology, and the circumstances of the event.

This workshop will focus on the investigative applications and best practice model of Practical Homicide Investigation® and the medicolegal evaluation of specific equivocal cases as well as the application of forensic pathology to the investigative process to accurately determine the cause and manner of death through a multidisciplinary medicolegal investigation as they relate to the investigation of equivocal deaths and offender-manipulated homicide scenes. The presentation will also feature cases that involve juveniles who commit murder with a level of sophistication and planning well beyond the age of the offender as well as aquatic death investigations involving children and adults.

Upon completion of this workshop, attendees will be familiar with the importance of crime scene integrity, the management of the homicide investigations, and the processing of the homicide crime scene as well as the application of the medicolegal investigation specifically as it relates to injury causation and the cause and manner of death.

Forensic Science, Equivocal Death, Aquatic Death

W11 Child Homicides: The Critical Role of Interdisciplinary Expert Collaboration

Evan Matshes, MD, Academic Forensic Pathology Inc, 6927-48th Street, SE, Ste 200, Calgary, AB T2C5A4, CANADA; Michael Cavilla, BA*, Calgary Police Service, Major Crimes Section, Calgary, AB, CANADA; Chris Milroy, MD, LLB*, Ottawa Hospital, 501 Smyth Road, Box 117, 4th Fl CCW, Ottawa, ON K1H 8L6, CANADA; and Jacqueline L. Parai, MD*, Ottawa Hospital, Division of Anatomical Pathology, 501 Smyth Road, Box 117, 4th Fl, Ottawa, ON K1H 8L6, CANADA*

After attending this presentation, attendees will better understand the main types of pathology seen in child abuse cases, the problems of identifying time sequences, how injuries are inflicted based upon the pathology present, and how law enforcement agencies can use medical and scientific evidence to investigate allegations of child homicide. Attendees will also learn how evidence is presented and challenged in homicide trials in the Canadian criminal justice system and how the Canadian legal system has dealt with undercover police operations and their admissibility as evidence.

This presentation will impact the forensic science community by providing information on different law enforcement techniques and how forensic medical evidence can be integrated into a complex investigation of a difficult and often contested area of the criminal justice system.

Sudden, unexpected, and/or violent death in childhood is uncommon to rare beyond infancy. Death investigators, forensic pathologists, law enforcement officers, and others faced with the investigation of such fatalities may be challenged by various factors including: (1) an incomplete, changing, or non-factual history of the circumstances of death; (2) complex pathology that cannot easily be interpreted within the vague historical data; and, (3) the reality that some injuries can be explained by non-criminal, unusual, bizarre, or rare activities of daily living that may initially seem implausible.

Well-documented public failures in pediatric forensic pathology have resulted in a shift in classic child death investigation. That is, while pathologists were often taught to "think dirty" when a child dies, modern wisdom has led to a shift toward "thinking truth." Without any doubt, all individuals involved in a child death investigation have a desire to reach factual, defensible conclusions and, within their roles, serve the public that pays for their services. Pediatricians, child protection services members, and law enforcement officers are, in general, advocates for "victims," and in that role, have distinct functions meant to protect other children and the public from real or potential harm. Forensic pathologists are in a somewhat unique role in that they are not advocates for any victim, person, family, or agency. Rather, they are advocates for science. It is this delicate relationship between "advocates" and the "non-advocate" forensic pathologist that will be explored in detail through case study, emphasizing the powerful partnership that can be developed through interdisciplinary collaboration, including, importantly, the use of so-called "hold-back evidence."

This presentation will review the modalities of death in the first decade of life (beyond infancy), with a focus on blunt trauma. Following this Socratic-style review, the presentation will shift to a single case study, the death of MJ, a 6-year-old girl who was found unresponsive at home with multiple injuries, and who later died in the hospital. The case study will revolve around the crucial and delicate relationship between a consulting forensic pathology expert and law enforcement officers, and will include a formal discussion concerning the oblique role for forensic pathology in a so-called "Mr. Big" undercover operation — a Canadian technique used in a number of different types of crime.

The independence of forensic pathologists, coroners, and medical examiners from the criminal justice system is a fundamental tenet of death investigation. When political and other influences are allowed to color medical opinions, a "truth seeking" paradigm cannot exist. That said, beyond the data sharing typical among agencies that investigate death, collaboration between an "expert" forensic pathologist and an investigative agency can promote and enhance the truth-seeking model and promote accuracy within the entire "system" of child death investigation.

Child Homicide, Expert Evidence, Undercover

W12 Development of a Reasonable Minimum Documentation Standard for Latent Prints

Heidi Eldridge, MS, RTI International, 3040 E Cornwallis Road, RTP, NC 27709; and Jeri D. Ropero-Miller, PhD*, RTI International, 3040 Cornwallis Road, PO Box 12194, Bldg 7, Rm 211, Research Triangle Park, NC 27709*

After attending this presentation, attendees will have: (1) hands-on experience in the difficulties of creating a one-size-fits-all policy for assigning documentation requirements to latent print evidence based upon the difficulty of the image; (2) experiential first-hand knowledge of some of the key attributes that factor into latent difficulty classifications; and, (3) a better understanding of the arguments for enhanced documentation as well as some practical suggestions on how to implement such a policy and the tools to design a policy that conforms to their own agency's operational needs.

This presentation will impact the forensic science community by furthering the dialogue surrounding the need for and implementation of realistic documentation policies while providing attendees with the practical tools and advice necessary to successfully craft and implement needed minimum documentation standards for latent print comparison work policies in their own laboratories.

Critics, courts, and researchers alike have been clamoring for increased documentation requirements for latent print comparison work. Accreditation standards support it and good scientific practice requires it, yet surprisingly few forensic laboratories even have a minimum documentation policy. Typically, the amount of documentation performed is either minimal in the extreme or is left entirely to the discretion of the individual examiner.

It seems that many laboratories incorrectly assume that taking the time to document will encompass a large volume of additional work without any measurable benefit, while it also seems to be that laboratories simply don't know how to accomplish designing and implementing such a policy. Part of the problem is that a sensible documentation policy should be predicated on the difficulty of the images in question — easy latents should require very little documentation, while more difficult latents should be subject to enhanced documentation. But as there are no generally accepted criteria for defining complex prints, it becomes difficult to determine when to apply these different policies.

This presentation will begin with a lecture on the philosophy of documentation — why do we document? Who are we doing it for? What makes it good scientific practice? Next will be exercises looking at actual latent prints and making quick, gut-reaction determinations about the quality of each image. Each image will be sorted into one of three categories based on perceived quality level and consensus among participants will be undertaken on these determinations.

Following the first exercise, the attributes of a latent image that make it more or less difficult will be discussed, and some visual training on what each attribute looks like will be reviewed in order to minimize variability between analysts in interpreting the criteria.

In a second exercise, participants will grade a set of latents according to the attributes that each displays. The results of this exercise will be reviewed as a group, once again determining what type of consistency can be reached among the participants.

Once the exercises are complete, the presentation will return to philosophy, with participants engaging in a roundtable discussion to identify useful features of a documentation policy and what are seen as the potential challenges to implementation.

Finally, suggestions will be offered for policies that could be implemented that will fulfill the goals of documentation, while having as small an impact on operations as possible.

The National Institute of Justice (NIJ) Forensic Technology Center of Excellence (FTCoE) is committed to improving the practice of forensic science and strengthening its impact to agencies dedicated to combating crime. This FTCoE workshop recognizes the importance of balancing the implementation of best practices with recognizing the operational needs of a functional forensic science laboratory.

Documentation, Latent Prints, SOPs

W14 Vaping: What You Didn't Know About Electronic Cigarettes — And Why You Should Care

Michelle R. Peace, PhD, VA Commonwealth University, 1015 Floyd Avenue, PO Box 843079, Richmond, VA 23284; Justin L. Poklis, BS*, Virginia Commonwealth University, Dept of Pharmacology & Toxicology, 410 N 12th Street, Rm 746, PO Box 980613, Richmond, VA 23219-0613; Richard N. Dalby, PhD*, University of Maryland, School of Pharmacy, 20 N Pine Street, PH N309E, Baltimore, MD 21201; Matthew R. Wood, MS*, Ocean County Sheriff's Dept, Forensic Science Laboratory, Toms River, NJ 08753; and Adam Polhemus, BA*, New Jersey State Police, PO Box 7068, West Trenton, NJ 08628*

After attending this presentation, attendees will be able to: (1) understand the mechanism and advantage of aerosols as a drug delivery system; (2) articulate the history of electronic cigarette development, their operation, and their components; (3) understand how electronic cigarettes are manipulated for abuse; and, (4) describe an analytical approach for e-cigarette components and e-liquid formulations to include real casework involving electronic cigarettes.

This presentation will impact the forensic science community by increasing awareness of electronic cigarette use as an emerging and popular drug of choice and the abuse trend leading to an international criminal justice concern. This presentation will also provide a foundation by which controlled substances units, forensic toxicologists, death investigators, and medical examiners can develop analytical methodologies and refine interpretative opinions when electronic cigarettes are used as a Route Of Administration (ROA).

Electronic cigarettes (e-cigarettes or e-cigs), known as "Personal Vaporizers" (PV) by avid users or Electronic Nicotine Delivery Systems (ENDS) by the industry, have experienced a significant increase in popularity for those seeking an alternative to smoking traditional tobacco products. These products are comprised of a battery-powered atomizer and a cartridge filled with a pharmaceutical (nicotine), flavorings, and water dissolved in glycerol products. E-cigarette devices are manufactured with a spectrum of personalization opportunities such as off-the-shelf non-customizable devices, customizations such as self-wrapping of the element, homemade wicks, self-preparation of the e-cigarette liquid formulation, cups to hold plant material, dripping vs. wicking, and wattage adjustors to administer a desired drug dosage.

The lack of enforced regulation has made e-cigarettes easy to access and has shepherded the nefarious use of electronic cigarettes. The use of the electronic cigarette as an illicit drug delivery device is touted on websites, forums, blogs, and videos describing how best to use them for specific illicit drugs such as tetrahydrocannabinol, methamphetamine, fentanyl, and heroin. They also explain at length the benefits of "vaping" illicit drugs as it can be done in public without question (there is no odor and vaping is not just acceptable, it is "cool").

Analyzing paraphernalia for drug usage is a practiced and conceivably straightforward methodology established in controlled substance laboratories nationwide; however, electronic cigarettes are still largely uncharacterized. Little is known or understood about their construction, let alone how they are potentially used to deliver illicit drugs. Additionally, from a toxicological perspective, little is documented regarding the delivery of nicotine, particularly as a function of power, for electronic cigarettes. Additionally, even less is known regarding the adulteration of electronic cigarettes and how the e-cigarettes are used or modified to optimize the delivery of an adulterant. Few peer-reviewed manuscripts exist in the literature that describe, define, and illustrate the use of electronic cigarettes.

This presentation will describe how electronic cigarettes work and their efficacy in drug delivery. Given that one of the roles of the forensic scientist is to define and characterize drug usage trends, this is being recognized as a relevant and identified threat to public health and criminal justice. This presentation will increase insight into the analytical efforts in controlled substances and in interpreting the findings and opinions of scientists, medical examiners, death investigators, and forensic toxicologists as related to electronic cigarettes. Attendees will also be more aware of the nature of drug usage, abuse, and overdose cases in which electronic cigarettes were used to deliver an illicit drug.

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Electronic Cigarettes, Controlled Substances, Toxicology

W15 Addressing Damaged Mobile Devices for Data Acquisition

Steven B. Watson, BA, 4377 W 117th Court, Westminster, CO 80031; Samuel I. Brothers, BBA*, US Customs & Border Protection, 7501 Boston Boulevard, Rm 113, Springfield, VA 20598; and Richard Ayers, MS, 100 Bureau Drive, MS 8970, Gaithersburg, MD 20899-8970*

After attending this presentation, attendees will have: (1) explored the topic of damaged mobile devices; (2) reviewed the existing literature in this and peripheral research areas; (3) completed hands-on activities, including the examination of a ballistics-damaged mobile phone, the documentation of the damage to the device in a written report and with photo documentation, and the disassembly of a ballistics-damaged mobile phone; and, (4) employed a donor device via the "fraternal clone" method to repair the device for power on and data acquisition.[1] Attendees will affect the future direction of the damaged devices research by providing input into the damaged devices program.

This presentation will impact the forensic science community by establishing that simply because the device is damaged, this does not mean the data is gone. Successful data acquisition is possible from damaged mobile devices. While each of the damaged device focus areas has the potential for catastrophic damage to the intact electronic device, the potential of data residing on the embedded flash memory of these devices still exists and presents a new research area with limited scientific research in the field of digital forensics.

This presentation reviews a series of research projects that involved inflicting damage to mobile devices with scientific precision, then documenting the damage and remediation with the intention of publishing the results to the digital forensics community. The scope of the damaged devices projects includes liquid damage, thermal damage, impact damage, and ballistics damage.

Most agencies do not receive severely damaged mobile devices frequently enough to develop solid expertise in dealing with these devices. Early survey input suggests that most agencies receive a water-damaged device only once per annum. Introductory research has been completed related to blood-damaged mobile devices and draft compilation of best practices from the Scientific Working Group on Digital Evidence.[2,3] This presentation will review existing literature in the digital forensics community and other peripheral research areas, highlighting the integration of current results and findings from the initial projects into subsequent projects.

Severe damage to mobile devices potentially affecting the ability to extract data can be caused by a number of factors. Aqueous solutions cause damage through galvanic and electrolytic corrosion to the internal components of electronic devices, the hygroscopic tendencies of the printed circuit board materials causing myelination of the circuit board layers, and chemical damage introduced by the chemical properties of the aqueous solution. Thermal damage causes plastic components on the exterior and interior of the devices to melt or burn, burning of insulating layers, circuit boards, and batteries, and even melting of the solder connections that connect the circuitry of the mobile device. Impact damage from high-velocity impacts cause screens and cases to break or shatter, batteries to begin leaking, and components to be knocked off the circuit board. Ballistics damage from high-velocity projectiles or explosive materials causes catastrophic damage to areas of the device or, in some instances, complete disassembly of the device.

This damaged devices workshop seeks to ask the questions, explore the answers, and provide real-time guidance to the field on addressing damage mobile and embedded devices.

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2. Dudeck K.C., Brennan T.C., Embury D.J. Decontamination of blood soaked electronic devices using ultrasonic technology. *Forensic Science International*, vol. 214, pp. 88-95, 2012.
3. Scientific Working Group for Digital Evidence. *SWGDE Best Practices for Handling Damaged Mobile Devices.* (Draft) USA:SWGDE, 2014.

Damaged Mobile Devices, Damaged Mobile Phones, Water Damaged Devices

W16 The American Academy of Forensic Sciences (AAFS) Humanitarian and Human Rights Resource Center

Douglas H. Ubelaker, PhD, Smithsonian Institution, Dept of Anthropology, NMNH, MRC 112, Washington, DC 20560; Morris V. Tidball-Binz, MD*, 4 Chemin des Fleurs, Ferney Voltaire, L'Ain 01210, FRANCE; Marilyn A. Huestis, PhD*, Chemistry & Drug Metabolism, Intramural Research, NIDA, NIH, 251 Bayview Boulevard, Rm 05A721, Baltimore, MD 21224; Sabra R. Botch-Jones, MS, MA*, Boston University School of Medicine, Biomedical Forensic Sciences, 72 E Concord Street, Boston, MA 02118; Dawn M. Mulhern, PhD*, Fort Lewis College, Dept of Anthropology, 1000 Rim Drive, Durango, CO 81301; Luis Fondebrider, PhD*, Rivadavia 2443, 2do Piso, Dpto.3 Y 4, 1034 Capital Federal, Buenos Aires, ARGENTINA; Michael S. Pollanen, MD*, Ontario Forensic Pathology Service, 25 Morton Shulman Avenue, Toronto, ON M3M 0B1, CANADA; Duarte Nuno Vieira, MSc, PhD, MD*, Rua Antonio Jose de Almeida, No 117, Coimbra 3000-044, PORTUGAL; and S. Cordner, MB*, Victorian Institute of Forensic Medicine, 57-83 Kavanagh Street, Southbank, Victoria 3006, AUSTRALIA*

After attending this presentation, participants will understand the structure of the new AAFS Center and current developments. Attendees will also be informed about key issues and applications within different global regions.

This presentation will impact the forensic science community by raising awareness of the new AAFS Center and important developments in global applications of humanitarian and human rights forensic science.

The value of the application of forensic science to humanitarian and human rights cases is growing as research expands, increasing the importance of the inclusion of such cases in education and practice. This presentation will provide current information on the development, structure, and progress of the new AAFS Center and its subcommittees, which will promote the application of contemporary forensic science to humanitarian and human rights cases around the world. In addition to providing access to AAFS resources to human rights cases, the Center will also offer support to AAFS members applying their skills to human rights projects, encouraging involvement in such instances. Perspectives from committee members will outline how the Center fits in with global advances and helps address issues relating to humanitarian and human rights forensic science.

Attendees will better understand the challenges faced and the benefits to be attained from further development and research in this area. Presenters from diverse research backgrounds will showcase how the new Center and its developments can fill a variety of niches in different contexts around the world. These perspectives emphasize the Center's potential for utilization in diverse situations and integration into scholarship. The Center will be comprised of an advisory committee, which will review proposals for support, and four subcommittees: Publications and Documents, Laboratory and Analysis Protocols (LAP), and Education, and Equipment. Additionally, many members have responded with their CV and personal letter of interest in participation as the Center advances. A database has been compiled of these individuals with the understanding that they may be contacted regarding circumstances related to their interests, specialties, and skills. This database and the committees are comprised of individuals from various regional backgrounds who represent all 11 sections of the AAFS.

The AAFS Humanitarian and Human Resource Center seeks to unify the resources of the AAFS to provide assistance for the growing demand of forensic science applications to human rights cases. It also seeks to encourage the integration of human rights issues into contemporary forensic science education as the field continues to expand.

Human Rights, Humanitarian, Resource Center

W17 Postmortem Monocular Indirect Ophthalmoscopy (PMIO)

Patrick E. Lantz, MD, WFU School of Medicine, Dept of Pathology, Medical Center Boulevard, Winston-Salem, NC 27157-1072; and Candace H. Schoppe, MD*, Southwestern Institute of Forensic Sciences, 2355 N Stemmons Freeway, Dallas, TX 75207*

After attending this presentation, attendees will be able to: (1) differentiate between direct and indirect ophthalmoscopy, noting advantages and limitations of each technique for the postmortem detection of fundal hemorrhages; (2) discuss the fundal location of retinal hemorrhages relative to their projected aerial image during monocular indirect ophthalmoscopy; and, (3) accurately draw retinal abnormalities observed during monocular indirect ophthalmoscopy with a simple ocular model on a fundal diagram.

This presentation will impact the forensic science community by providing an overview of PMIO, facilitating skill acquisition, and evaluating practical training and image acquisition with a smartphone.

Postmortem examination of the retina has relied on ocular evisceration. In most medical examiner/coroner jurisdictions, ocular enucleation is not a standard autopsy procedure unless child abuse is suspected, thus creating observational bias when citing the prevalence of postmortem fundal findings such as retinal hemorrhages (preretinal, flame-shaped or splinter, and dot/blot), perimacular retinal folds, retinoschisis, and postmortem artifactual retinal folds. PMIO permits examination of the decedent's posterior fundus and portions of the peripheral retina. The required equipment necessary for PMIO is relatively inexpensive and, when compared to direct ophthalmoscopy, the technique is less affected by corneal clouding, lens opacity, or vitreous hemorrhage and offers a wider field of view. PMIO uses a focal light source and an aspheric, convex condensing lens. An excellent source of coaxial illumination is a halogen or xenon surgical or procedural headlamp. This light source creates a collimated beam of light and permits the examiner to stabilize the condensing lens with both hands. Current aspheric lenses range from +14 to +40 diopters and are available in different diameters, permitting a field of view of 35°-55°. Postmortem corneal opacity may cause the fundus to appear hazy; however, by gently removing the epithelial layer of the cornea, the emergent image is usually of adequate quality to readily detect lesions such as fundal hemorrhages and retinal folds.

Learning how to perform and become proficient at PMIO can be perplexing and intimidating. Most pathology residents and forensic pathology fellows have limited exposure to indirect ophthalmoscopy. Because the projected aerial image is inverted and laterally reversed, precise descriptions or recording of fundal abnormalities can be challenging. Unlike binocular indirect ophthalmoscopy with a teaching mirror attachment, a procedural headlamp worn by the instructor does not permit the fellow or resident to view the projected aerial image simultaneously during PMIO. To address these learning obstacles it is necessary to develop tools and models to facilitate skill acquisition. Most smartphones can capture the image formed during indirect ophthalmoscopy using the smartphone's light source to illuminate the fundus. An hour or two with an inexpensive ocular model can shift the learning curve of the resident, fellow, or forensic pathologist substantially to the right in how to correctly position the light source and hold the indirect lens.

This presentation consists of an initial discussion and presentation that reviews the technique of PMIO, highlighting the optics, the equipment, and examples of abnormal fundal findings found at autopsy by PMIO and the use of a smartphone to capture the projected aerial image. Next, attendees will have a realistic learning experience by practical hands-on training with a procedural headlamp, an aspheric indirect lens, and a simple ocular model containing a variety of retinal abnormalities observed at autopsy. The ocular models have variably sized "pupillary" openings and some will have clear acetate over the openings to simulate corneal glare. Attendees will receive assistance in positioning the procedural headlamp, holding the indirect lens, viewing the projected aerial image, and accurately recording the retinal abnormalities. Attendees with smartphones can practice still image acquisition and video recording of fundal images produced by PMIO. Attendees will learn how to hold the smartphone with one hand while imaging the fundus and how to use a mini-tripod so the condensing lens can be stabilized with both hands, thus enhancing image stabilization and acquisition.

Following practice visualizing, diagramming, and image capture techniques of numerous fundal images, attendees have the option of being evaluated with a series of unknowns. Self-assessment of technical skill training and review of the unknown retinal findings concludes the presentation. As part of the presentation, attendees will be given a USB thumb drive with the introductory presentation, sample retinal images, fundal diagrams, and articles on PMIO.

Indirect Ophthalmoscopy, Retinal Hemorrhage, Smartphone

W18 Improving Your Image: How to Get the Best Out of Your Expensive X-Ray Equipment

Gerald J. Conlogue, MHS, Quinnipiac University, Diagnostic Imaging Program, 275 Mount Carmel Avenue, Hamden, CT 06518; and Mark D. Viner, MSc*, Cranfield Forensic Institute, Defence Academy of the UK, Inforce Foundation, Shrivenham SN6 8LA, UNITED KINGDOM*

After attending this presentation, attendees will: (1) have a better understanding of basic imaging principles using either film or a digital recording media; and, (2) incorporate these fundamentals into image optimization.

This presentation will impact the forensic science community by providing attendees with a better understanding of the association of basic radiographic principles, image acquisition, and optimization of image quality. The target audience for this presentation would include any individual involved in acquiring radiographs in a forensic setting. This would include, but is not limited to, medical examiners, forensic pathologists, dentists, anthropologists, autopsy technicians, and radiographers.

Medical imaging equipment and practices have advanced dramatically in the past two decades; however, due to the rapidly developing technology, many of the practices have not been adapted into forensics. The presentation delivery teams have had extensive experience in integrating technical advances with image optimization in a variety of settings including medical, anthropological, and forensics areas.

The Quinnipiac University Team has served as consultant to the Office of the Chief Medical Examiner for the State of Connecticut since 2002. During the academic semester, they not only radiographed victims but also trained the autopsy technicians on basic imaging fundamentals. Until the spring of 2014, all images were acquired using film, but since that date, the team has assisted in the transition to Computed Radiography (CR). In 2012, the team acquired valuable knowledge dealing with mass causalities from its experiences with the victims from the incident in Newtown, CT. In addition, the team has demonstrated alternative specimen imaging approaches utilizing industrial radiographic film, Multi-Detector Computed Tomography (MDCT), and tomosynthesis.

The Forensic Institute at Cranfield University/Inforce Foundation Team has extensive experience in delivering training in forensic radiography and mass fatality incidents. They deliver an annual Masters Level course for radiographers, anthropologists, and investigators and organize regular training and exercising for the United Kingdom Forensic Radiography Response Team. Team members have experience in routine Medical Examiner (ME) office work, cold case investigations, and many mass casualty incidents, including the 2007 London suicide bombings, Southeast Asia Tsunami, and investigations into genocide and human rights abuses in the former Yugoslavia, Sierra Leone, and Rwanda as well as in examination of archaeological remains. They provide advice on forensic imaging and emergency planning to the United Kingdom Government, United Nations International Criminal Court.

This presentation will begin with a general discussion of forensic imaging protocols that will include routine cases, cold case review, and mass casualty incidents in the United Kingdom and Connecticut. Although the use of film as an image receptor is declining, there are still locations where it is employed today. Several advantages of film will be discussed along with all the factors that must be considered with image optimization for this recording media, such as the formulation of a technique chart. In addition, the basic principles of film processing will be reviewed and will include consideration of automatic processing equipment and optimum operating conditions. An overview of the two types of digital image recording systems, direct Digital Radiograph (DR) and Computed Radiography (CR), will be reviewed. The advantages and disadvantages of each will be considered. Once the equipment basics have been discussed, the presentation will move on to methods and procedures to acquire images for routine and non-routine situations.

Forensic Radiography, Radiographic Film, Computed Radiography

W19 Diversity and Inclusion at the Forensic Science Workplace

Nikolas P. Lemos, PhD, OCME, Forensic Lab Division, Hall of Justice, N Terrace, 850 Bryant Street, San Francisco, CA 94103; Daniel S. Isenschmid, PhD*, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090; Chinyere M. Williams, BS*, 2527 8th Avenue, Apt 211, Oakland, CA 94606; and Cathy Tobin*, Cathy Tobin, PHR,525 First Avenue, W, Apt 210, Seattle, WA 98119*

After attending this presentation, attendees will be equipped with the necessary knowledge and tools to reflect on the past, put it into the context of the present, and create the conditions for an inclusive and diverse work environment.

This presentation will impact the forensic science community by helping attendees and their organizations better prepare to meet the evolving needs of a diverse and inclusion-focused workplace through the use of emerging trends, data, and practical application methodologies.

Diversity means understanding that each individual is unique and recognizing our individual differences. These can be along the dimensions of race, ethnicity, gender, sexual orientation, socio-economic status, age, physical abilities, religious beliefs, political beliefs, or other ideologies. It is the exploration of these differences in a safe, positive, and nurturing environment. It is about understanding each other and moving beyond simple tolerance to embracing and celebrating the rich dimensions of diversity contained within each individual. Inclusion puts the concept and practice of diversity into action by creating an environment of involvement, respect, and connection — where the richness of ideas, backgrounds, and perspectives are harnessed to create workplace, education, or business value.

All organizations need both diversity and inclusion to be successful. Companies with diverse workforces and leadership consistently outperform other companies and it is now more common than ever before for companies to routinely include in their workforce chief diversity officers.

The practice of diversity and inclusion reflects much of what is happening in the United States and the global workforce — it is in a state of constant evolution and flux.

Change is an expectation of the up-and-coming workforce. We, as forensic science educators, researchers, and practitioners, must drive and embrace this change and continue to create a more inclusive, healthier, and engaging forensic science workplace. The American Academy of Forensic Sciences has led the way and continues to encourage and support the membership's effort for a diverse and inclusive forensic workplace.

As workforces continue to become increasingly global, the need to understand and practice diversity and inclusion is ever more pressing.

This program will briefly review the historical perspectives of how societies and organizations have responded to, and were shaped by, human differences. Science has historically acted as an impetus for the political and social action needed to move diverse groups from segregation into mainstream society. The scientific community is often a conduit of change and it is that legacy that compels us to proactively raise awareness and create an inclusive and diverse environment that serves both our employees and the communities in which they live and work.

This presentation will provide tools that bolster diversity and inclusion in the forensic science workplace in order to improve our ability to raise awareness and create the conditions for an inclusive and diverse environment. This presentation will also demonstrate the existing connections between diversity, inclusion, and productivity and examine the nature of privilege in the workplace and how it negatively impacts employee engagement and organizational performance.

Attendees will be offered methods of identifying bias, whether positive, negative, seen or unseen, and will learn best practices for mitigating the all-too-often negative outcome of biases as well as preventing those outcomes by stopping bias at its source. Finally, the presentation will provide attendees with practical techniques in order for them to return to their workplaces and take action.

Diversity and Inclusion, Forensic Science, Workplace

W20 On the Leading Edge of Forensic Science

Zeno J. Geradts, PhD, Netherlands Forensic Institute, Laan van Ypenburg 6, Den Haag, SH 2497 GB, NETHERLANDS; Laura L. Liptai, PhD*, BioMedical Forensics HQ CA/FL, 1660 School Street, #103, Moraga, CA 94556; Robert M. Thompson, BS*, NIST, Special Programs Office-Forensic Sciences, 100 Bureau Drive, MS 8102, Gaithersburg, MD 20899; Katrin Franke, PhD*, Gjovik University, Teknologivegen 22, Gjovik, NORWAY; Arian C. van Asten, PhD*, Netherlands Forensic Institute, Laan van Ypenburg 6, The Hague, Zuid Holland 2497GB, NETHERLANDS; Gwyn Winfield, MA*, CBRNE World, 1 Winnall Valley Road, Ste 26, Falcon Communications, Winchester, Hampshire S023 0LD, UNITED KINGDOM; Victor W. Weedn, MD, JD*, George Washington University, 2100 Foxhall Road, NW, Somers Hall, Lower Level, L-12, Washington, DC 20007; Edward G. Bartick, PhD*, George Washington University, Dept of Forensic Sciences, 2100 Foxhall Road, NW, Washington, DC 20007; Mehdi Moini, PhD*, George Washington University, Dept of Forensic Sciences, 2100 Foxhall Road, NW, Washington, DC 20007; and Matthew T. Henshon, AB, JD*, Henshon Klein LLP, 120 Water Street, Boston, MA 02109*

After attending this presentation, attendees will better understand new developments in forensic science that may have impact on their work.

This presentation will impact the forensic science community by providing an overview of some of the new developments in forensic science and by opening a forum for the discussion of issues that arise regarding such developments. A wide variety of developments that will soon impact forensic science have been identified within the Think Tank Committee of the Forensic Sciences Foundation, Inc.

The development of drones and the forensic issues concerned with finding digital traces is seen as one of the topics. The use of drones in forensics is also one of the topics, especially as the methods have become much more attractive due to a drop in prices and increased availability.

The forensic discipline of firearm and tool mark identification currently relies on the optical micro-comparison of features that were produced by movement or by the impression of tool-bearing surfaces. The comparisons are conducted side-by-side with comparison microscopy, incorporating the skill and artistry of the examiner to balance the tool mark surface positions, angles, and illumination. If sufficient agreement is observed, a subjective determination of identity may be concluded. While proven to be accurate with a low false positive error rate, there has been renewed interest in objective comparison methods that are based on the actual 3D measurements of tool mark surfaces that are compared using statistical methods to objectively "measure" the similarity of the two surfaces. This presentation will review the most recent emerging technologies in surface topography measurements, their adaptation to firearm and tool mark analysis, and recent research in the objective measurement of similarity.

Another important topic is the investigation within a Chemical, Biological, Radiological, and Nuclear (CBRN) crime scene as the interrogation of CBR agents presents a variety of problems. Primary among those at the scene is an intense degree of political scrutiny and a high thermal burden. How do you accurately take high value samples when you are in a Level A "spacesuit"? How do you know where they are and what should you prioritize in the 20 minutes of air that you will have on the scene? The European Commission Generic Integrated Forensic Toolbox (GIFT) is answering these questions and can share some of the data.

The capabilities and usage of field instrumentation are growing rapidly. While these instruments do not have the versatility of laboratory instruments, they are being designed to carry out specific critical *in situ* field tasks that save time and money and reduce laboratory analyses. For example, hand-held Raman has been used for the detection of organic compounds such as controlled substances; however, since the detection is based on spectroscopic techniques, the results are preliminary and only good for screening purposes — findings still need to be analyzed by confirmatory laboratory techniques such as separation followed by mass spectrometry. To address this need, the George Washington University is developing a hand-held, ultra-fast capillary electrophoresis mass spectrometry for on-site, real-time analysis of chemical and biological compounds with optical isomer separation capability.

By allowing forensic analyses to be performed in real time at the crime scene or nearby facilities, valuable forensic information can be provided at the beginning of the investigation and can thus increase the quality of law enforcement. By introducing robust, easy-to-use portable forensic technology, the efficacy of the criminal justice system can be improved; however, such benefits can only partly be accomplished when the results are of an indicative nature and detailed analyses at a forensic laboratory remain necessary. The full potential of real-time forensic investigations can only be realized when the results can also be used as evidence in court. The Netherlands Forensic Institute (NFI) recently published a vision on integrated forensic platforms to merge the speed provided by real-time forensic analysis with the quality standards of accredited laboratory methods. Currently, the NFI is working on several such dedicated platform solutions that would enable DNA profiling, the chemical identification of illicit drugs, and the study of large amounts of digital evidence. This technological revolution could lead to a new role for forensic institutes in which forensic experts are focused on designing, developing, and maintaining forensic platforms, allowing other professionals in the criminal justice system to examine the physical, chemical, biological, and digital evidence. Forensic institutes could then focus their usually scarce expert capacity to interdisciplinary investigations in complex cases and forensic intelligence.

New Developments, Robotics, Automation

W21 Crime Assessment: Solving Crime Beyond Profiling

Richard D. Walter, MA, 1879 Chenango Street, Montrose, PA 18801; Klaus C. Neudecker, MD*, Schirmgasse 268, Landshut, Bavaria D-84028, GERMANY; Patrick Zirpoli*, 149 Spruce Swamp Road, Milanville, PA 18443; Amanda L. Farrell, PhD*, Marymount University, School of Education and Human Services, 2807 N Glebe Road, Arlington, VA 22207; and Lurena A. Huffman, BS*, Suffolk Police Department, 23 N Greenfield Avenue, Hampton, VA 23666*

After attending this presentation, attendees will understand that crime assessment is a method of crime investigation that utilizes key structures within the criminological continuum to examine the presence and/or absence of evidence found at the crime scene. Within this framework, there are four major classifications, referred to as the subtypes hereafter, which will be introduced and explained. These subtypes span the criminal spectrum and manifest in the expression of pathological constellations behaviors that can be recognized. Predicated upon these primary factors and coupled with additional principles, attendees will be able to grasp that understanding the crime scene through the crime continuum provides a critical understanding for the motives, methods, and opportunities of the crime.

This presentation will impact the forensic science community by providing attendees with the understanding that, although the human experience is variable, crime patterns can be coded to reveal interlocking and separate vectors. By doing so, recurrent elements and themes are developed to group common factors for various desires, intentions, and plans. Ergo, dependent upon the intended outcome, the crimes can reveal differentiated power and anger issues, levels of intimacy, and necessary idiosyncrasies that must be avoided. Accordingly, while acting out crime, the criminal many times inadvertently leaves these pre-crime, crime, and post-crime clues for investigators to find and analyze.

Historically, the work associated with profiling has utilized the psychological continuum to project clinical diagnosis and treatment to advise investigators on the *inference/meaning* of the evidence at the crime scene. Due to a lack of understanding of crime patterns, the traditional profiler may err by translating individual clinical data into the analysis of the crime patterns and meaning. By incorporating a *projective* psychological mythology into the crime continuum, the results will vary from minor errors to major contradictory flaws of evidence that may misdirect the investigation and/or judicial testimony.

In contrast to the risks associated with traditional profiling efforts, crime assessment measures the crime by known major subtype crime patterns (Power-Assertive type; Power-Reassurance type; Anger Retaliatory type; and Anger-Excitation type). These subtypes provide a structural foundation from which to analyze crimes, in effect becoming the DNA of crime. That is, the crime research has identified key elements of the crime which can shape the investigation and provide critical knowledge regarding the various elements of the crime, to include, but certainly not limited to, providing recommended methods of apprehension, interviewing strategies, and prosecutorial considerations. Most importantly, inasmuch as crime assessment is reflective in process, the investigators and experts can explain the process of the investigation without the perils of projection.

Note: This workshop will use many cases, videos, and discussion points to illustrate the conceptual and applied understanding of crime assessment. Given the nature of the material, it is not recommended for those persons who are sensitive and/or in some form of crisis.

Crime Assessment, Criminal Investigation, Offender Behavior

W22 Developing A Professional Code of Ethics in Digital Forensics

James R. Dibble, BS, 14606 N Gleneden Street, Spokane, WA 99208; Michael M. Losavio, JD*, Department of Criminal Justice, University of Louisville, Louisville, KY 40292; Keith W. Miller, PhD*, University of Missouri - St. Louis, 1 University Boulevard, 201 Education Admin Bldg, St. Louis, MO 63121; Marcus Rogers, PhD*, Purdue University, 401 N Grant Street, West Lafayette, IN 47907; Anthony Skjellum, PhD*, Auburn University, Dept of Computer Science and Software Eng, 345 W Magnolia, 3101 Shelby Center, Auburn, AL 36849-5347; Rhesa G. Gilliland, MS*, US Postal Inspection Service, Forensic Laboratory Services, 22433 Randolph Drive, Dulles, VA 20104-1000; and Kathryn C. Seigfried-Spellar, PhD*, Purdue University, Computer and Information Technology, 401 N Grant Street, West Lafayette, IN 47907*

After attending this presentation, attendees will be aware of issues that constitute a need to generate support for a unified professional code of ethics in digital forensics and will identify the steps necessary to establish such a code.

This presentation will impact the forensic science community by bringing together key stakeholders and representatives in the area of digital forensics, including academics, practitioners, and vendors to discuss the need for a professional code of ethics.

Almost every criminal and civil investigation now involves some form of digital evidence, yet we are a profession that lacks a clearly articulated, consensus-based code of ethics. In fact, it has been argued that without a code of ethics, the field of digital forensics cannot even *be* a "profession."[1]

Unlike some professions (e.g., legal, medical), digital forensics has neither a professional association on par with the American Bar Association (ABA) or the American Medical Association (AMA), nor a comprehensive code of ethics comparable to the ABA's *Model Rules of Professional Conduct* or the AMA's *Code of Medical Ethics*. Instead, multiple professional associations exist — some of which also provide certification for digital forensics professionals — such as the International Society of Forensic Computer Examiners (ISFCE), the International Association of Computer Investigation Specialists (IACIS), the Digital Forensics Association (DFA), and the American Society of Digital Forensics and e-Discovery (ASDFED), to name a few. A handful of these associations have established a set of professional ethical standards that members or certificate holders are expected to follow, but this is not the norm.

Sponsored by the National Science Foundation's Science, Technology, and Society (STS) program, a workshop was held in May 2015 to discuss the need for a professional code of ethics in digital forensics. Some of the core areas discussed at the workshop included: misrepresentation of digital evidence, misrepresentation of credentials, duty to verify/validate/test if the tools are operating as intended, duty to not exceed one's own knowledge, and a duty to uphold confidentially and privacy. In addition, there is a need to address conflicts of interest (e.g., confirmation bias, loyalty to employer, financial bias, hired guns). In fact, the Ethics Committee of the Council of Scientific Society Presidents (CSSP) reached out to members of the American Academy of Forensic Sciences (AAFS) in July 2015 to determine if there were examples of bias in terms of the acceptance of results based on source of funding. Although the CSSP is concerned with a specific conflict of interest, there exist similar concerns in the field of digital forensics with conflicts of interest, such as ethical dissent (e.g., acts of conscience, whistle-blowing) and professional neutrality (e.g., examinations are valid regardless of employer); however, unlike the CSSP, almost none of the existing codes have enforcement mechanisms in place to investigate allegations of unethical conduct by digital forensics professionals or sanctions for offenders if violations are uncovered.

Based on the recommendation of attendees at the Professional Ethics in Digital Forensics workshop, a larger workshop is sought at AAFS that will bring together key stakeholders and representatives in the area of digital forensics, including academics, practitioners, and vendors. The goals of the workshop are to raise awareness about these issues, to generate support for a unified professional code of ethics in digital forensics, and to identify the steps necessary to establish such a code.

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Code of Ethics, Digital Forensics, Profession

W23 Considerations for Implementing Next Generation Sequencing (NGS) Technologies Into a Forensic Laboratory

Kimberly S. Andreaggi, MFS, ARP/AFDIL, 115 Purple Heart Drive, Dover AFB, DE 19902; Alice Briones, DO*, 599 Phillips Drive, Magnolia, DE 19962; Katherine B. Gettings, PhD*, NIST, 100 Bureau Drive, MS 8314, Gaithersburg, MD 20899; Erin M. Gorden, MFS*, Armed Forces DNA Identification Lab, 115 Purple Heart Drive, Dover AFB, DE 19902; Richard A. Guerrieri, MS*, 1 Corduroy Court, Stafford, VA 22554; Jennifer L. Higginbotham, MFS*, 115 Purple Heart Drive, Dover AFB, DE 19902; Christina M. Neal, MS*, Armed Forces DNA Identification Laboratory, 115 Purple Heart Drive, Dover, DE 19902; Walther Parson, PhD*, Muellerstraße 44, Innsbruck A-6020, AUSTRIA; Michelle A. Peck, MFS*, Armed Forces DNA Identification Laboratory, 115 Purple Heart Drive, Dover AFB, DE 19902; Joseph D. Ring, MS*, 115 Purple Heart Drive, Dover AFB, DE 19902; Peter M. Vallone, PhD*, 100 Bureau Drive, Gaithersburg, MD 20899-8311; Charla Marshall, PhD*, Armed Forces DNA Identification Laboratory, 115 Purple Heart Drive, Dover AFB, DE 19902; and Timothy P. McMahon, PhD*, Armed Forces DNA Identification Laboratory, 115 Purple Heart Drive, Dover Air Force Base, Dover, DE 19902*

After attending this presentation, attendees will understand NGS methodologies that can be applied to typical forensic specimens, as well as appreciate the considerations specific to the validation of NGS technologies.

This presentation will impact the forensic science community by discussing the benefits and challenges of implementing NGS into a forensic laboratory.

Over the past two decades, the gold standard for nucleic acid sequencing has been the chain-termination technique developed by Edward Sanger and colleagues in the late 1970s, now known as Sanger sequencing. In forensic laboratories, sequencing has historically been performed for mitochondrial DNA (mtDNA) typing, which is most applicable in cases with minimal quantities of nuclear DNA or in those lacking appropriate references for direct identification; however, there is strong forensic interest in the adoption of NGS technologies for wider use within the laboratory. NGS platforms enable massively parallel sequencing to generate millions of DNA sequence reads simultaneously. As such, NGS facilitates high-throughput DNA sequencing of multiplexed samples and DNA targets, which can be automated to streamline the laboratory workflow. Moreover, NGS data are quantitative and amenable to hands-off analysis within an expert system bioinformatic software package. This benefit thereby eliminates the need for visual assessment of electropherogram images that can slow the process of data review. The recent development of low-cost, high-throughput NGS platforms and commercial kits for forensic applications has made sequencing more accessible to forensic laboratories. Consequently, a demand has driven the forensic community to implement NGS technologies for routine use.

Forensic DNA laboratories around the world have begun the task of validating NGS technologies for missing persons and criminal casework as well as databasing efforts; however, these advances pose significant hurdles as traditional typing methods are traded for NGS assays, quantitation instruments, and sequencing platforms. First, NGS will require a transition from traditional length-based DNA typing methods to the sequencing of core forensic DNA markers. Consequently, sequence variation present in Short Tandem Repeats (STRs) will require an establishment of nomenclature for STR sequence analysis. NGS enhances the feasibility of entire mtDNA sequencing and enables Single Nucleotide Polymorphism (SNP) characterization as a feasible tool for genetic discrimination. In turn, the adoption of NGS sets the stage for ethical and legal discussions surrounding the use of phenotypic markers in forensics. Although other fields have adopted this technology successfully, forensic laboratories are beholden to strict guidelines and legal challenges that affect NGS implementation.

This presentation will provide a snapshot of the current progress of forensic DNA laboratories in the implementation of NGS technology. First, a historical perspective on DNA typing technologies will be presented to situate NGS within the context of methodological advancement. The presentation will follow with an overview of NGS methods available to the forensic community, and a discussion of laboratory infrastructure as it transitions to meet NGS requirements. Several presentations will focus on data generated from NGS workflows, including an evaluation of quantification systems as well as STR sequencing kits. The presentation will then turn to mitochondrial DNA sequencing, data analysis, and interpretation. Considerations surrounding the selection of NGS workflows and the challenges to the validation of NGS technology will be discussed. The final portion of this presentation will take the pulse of the broader forensic DNA community as it works to adopt NGS technologies in the laboratory.

The opinions or assertions presented hereafter are the private views of the author(s) and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the United States Army Medical Research and Materiel Command, or the Armed Forces Medical Examiner System.

DNA Analysis, Next Generation Sequencing, Validation

W24 Elder Abuse and Neglect: What's Happening to Grandma?

Amy Y. Carney, PhD, 210 Ivory Gull Way, San Marcos, CA 92078; Stewart D. Ryckman, MD*, 1468 Brookpark Drive, Mansfield, OH 44906; Debi Spencer, MFS*, CMR 467 Box 3364, APO, AE 09096; and Mark Carroll, BA*, Summit County Sheriff's Office, 53 University Avenue, Akron, OH 44308*

After attending this presentation, attendees will: (1) recognize the different forms of Elder Abuse (EA); (2) understand the motivation behind EA; (3) identify specific types of trauma found in EA; (4) recognize the injuries that may mimic trauma in the elderly; (5) understand the process of law enforcement response and death investigation in EA; and, (6) distinguish the similarities and differences between intentional neglect and self-neglect.

This presentation will impact the forensic science community by increasing the ability to detect the different forms of EA, distinguish abuse from neglect, and increase the awareness of the law enforcement response in cases of criminal abuse and death investigation.

The elderly, defined as those more than 65 years of age, are the fastest growing population in the United States, as well as in other countries around the world. By the year 2030, more than 20% of United States residents are expected to be age 65 and older, compared with 13% in the year 2010. By the year 2034, all of the baby boomers will be more than 70 years of age.

Elder abuse is a growing problem in the United States. Incidents of physical and sexual abuse, as well as neglect, continue to rise as the population ages. Maltreatment of the elderly is associated with increased morbidity and mortality, as well as increased health care costs. Fear, shame, and lack of knowledge contribute to underreporting of elder abuse and put the safety of elders at risk.

Definitions of elder abuse differ across the United States, as well as country to country, but usually include physical and financial abuse, verbal and emotional abuse, and neglect or potential neglect. Elder mistreatment can include both deliberate action or lack of action by a caregiver or family member and can occur in institutional or domestic settings.

It is this lack of agreement on the definition of elder abuse, as well as what constitutes elder abuse, that has made it difficult to assess incidence and prevalence. According to the World Health Organization (WHO), elder abuse is a single or repeated act, or lack of an appropriate action, that occurs in any relationship in which there is an expectation of trust and causes distress or harm to the older person. This definition excludes random acts of criminal behavior or violence, and it puts the trusting relationship at the center of the issue. It is this trusting relationship that often puts any resulting injuries into question, when physical signs of abuse are taken for the expected signs of aging, such as ulcers, bruising, or accidents, such as a fall. Injuries may be mistaken for result of disease or medication, and the possibility of abuse usually isn't considered.

In the 30 years since elder abuse was first identified as "granny battering," the medical and legal communities have come together to investigate elder abuse and add to the scientific knowledge in identification and intervention. Medical costs associated with violent injuries to elders in the United States are estimated at $5.3 billion dollars annually. This cost, as well as the morbidity and mortality associated with elder abuse, is expected to rise with the growth of the geriatric population.

The ability to identify abuse is the first step in assisting the elderly to safety. This presentation will assist attendees in recognizing the different types of elder abuse, understanding the motivation behind elder maltreatment, and provide specific case examples of abuse and neglect, which will assist the forensic professional in identifying and intervening in elder maltreatment. This presentation also provides tips and techniques for documentation, assists the forensic professional in distinguishing between accidental and criminal acts, and discusses the difficulties in prosecution in EA cases.

Elder Abuse, Neglect, Investigation

ANTHROPOLOGY

A1 Quantification of Radiologic Identification: Development of a Population Frequency Data Repository

Angi M. Christensen, PhD, FBI Laboratory, 2501 Investigation Parkway, Quantico, VA 22135; and Gary M. Hatch, MD*, University of New Mexico School of Medicine, Center for Forensic Imaging, MSC 07 4040, 1101 Camino de Salud, NE, Albuquerque, NM 87102*

After attending this presentation, attendees will be familiar with the development of a repository of population frequency data on radiologic features used in forensic identification comparisons.

This presentation will impact the forensic science community by allowing practitioners involved in radiologic comparisons to utilize population frequency data to enhance identification comparisons by providing statistical probabilities of correct and incorrect identification.

Radiographic comparison is a reliable means of personal identification in medicolegal death investigations. As with any identification approach (such as fingerprints, DNA, dentition, etc.), a key requirement of radiologic identification is that the trait or feature being compared in the antemortem and postmortem data must be relatively rare in the general population. The more unusual a shared feature is, the greater the probability that the identification is correct (i.e., that the two datasets originated from the same person). Population frequencies describe the frequency with which a feature is found in the general population, and they form the basis for the validity of any identification approach, including comparative radiology.

Most forensic radiology comparisons involve a relatively subjective assessment of the degree of similarity between antemortem and postmortem radiologic images (whether X-ray, Computed Tomography (CT), Magnetic Resonance Imaging (MRI), or other imaging modality), typically involving a qualitative visual comparison with the conclusion regarding an identification (or exclusion) being based on the skill and experience of the practitioner. Such assessments have been shown to be reliable in the sense that practitioners can locate matches (or pairs of images from the same person) among moderately large data sets, and misidentifications (or mismatches) have been demonstrated to be rare, with the ability to identify correct matches generally varying as a function of practitioner experience. However, subjective comparisons are insufficient for quantitatively assessing the strength (or evidentiary value) of an association and cannot typically be used to determine the probability of a correct (or incorrect) identification.

Rather than concluding that antemortem and postmortem radiologic images appear similar or the same, the results of radiologic identification comparisons are ideally expressed as likelihood ratios, which describe the probability of sharing radiologic features given that the identification is correct, over the probability of sharing the features if the identification is incorrect. The implementation of quantitative methods bolsters conclusions by providing statistical support for the probability of a correct (or incorrect) identification; however, quantitative methods require the acquisition and use of population frequency data for radiographic traits used in identification comparisons. In order to assess the probability of correct identification, the frequency of the trait shared in the antemortem and postmortem data must be known or estimated. Such data are currently absent for many features or difficult to access for others, precluding the use of quantitative methods in forensic radiologic identification in most cases. For certain skeletal/radiologic features, population frequencies may be reported in the journals and texts of disparate fields, often in publications unrelated to radiologic identification, making it difficult for forensic practitioners to locate the necessary data. For other traits, their population frequencies or variations in configuration require additional study.

In an effort to resolve this problem of insufficient or inaccessible data, this study is currently working to build a repository of estimated population frequencies for commonly assessed radiologic traits. Beginning with a thorough literature review, the first phase of the project involves mining currently available publications and data (including peer-reviewed medical and anthropological research and popular medical atlases) for documented population frequencies. The next phase of the project will involve the selection and analysis of additional traits using a large dataset of postmortem CT scans available through the New Mexico Office of the Medical Investigator. The database will eventually be made available through a publicly accessible website. Practitioners will then be able to reference the database, using the estimated population frequencies in forensic comparison casework. Researchers and practitioners wishing to participate in the data collection phase are encouraged to submit references or research to this study for possible inclusion in the database.

Forensic Radiology, Forensic Anthropology, Identification

A2 Systematic Bias in Estimating Body Mass of Korean Samples With the Morphometric Method of Ruff et al. (2005)

Yangseung Jeong, PhD, 419A Atkinson Drive, 905, Honolulu, HI 96814; and Eun Jin Woo, PhD, Seoul National University, Dept of Anthropology, San 56-1, Silim-dong, Kwanak-gu, Seoul, SOUTH KOREA*

After attending this presentation, attendees will appreciate the magnitude and cause of systematic bias in applying Ruff et al.'s morphometric method for body mass estimation to Asian samples, particularly to Korean samples.[1] Attendees will therefore be aware of the necessity of applying an adjustment factor that compensates for the bias prior to using this method for Asian populations.

This presentation will impact the forensic science community by quantifying potential errors associated with application of Ruff *et al.'s* morphometric method to Asian samples, and this research provides a theoretical basis for the bias.[1]

The morphometric method by Ruff *et al.*, a widely used body mass estimation method, is based on the cylindrical model.[1] The cylindrical model states that given specific density, the weight of a cylinder can be calculated from its height and breadth. For the morphometric method to have global applicability, the body composition of people (i.e., density of a cylinder) must be constant across populations; however, body composition differs between populations, particularly between Asians and non-Asians. It has been reported that the body fat percentage (BF%) of Asians is higher than of non-Asians with the same Body Mass Index (BMI) by 3%-5% points. Despite this difference, validation tests for this method have rarely been performed. In this research, the degree of potential bias was quantified when this method was applied to one population in Asia, Korean skeletal remains.

The body mass of 59 complete Korean male skeletons was morphometrically reconstructed using Ruff *et al.,* on which a regression equation was generated with the femoral head diameter.[1] Then, this new equation was applied to 54 Korean War casualties, whose estimated body mass was compared to the reported body mass of the Korean conscripts during the Korean War.[2] Although the 54 casualties were not identical to the individuals used in Park *et al.,* no significant discrepancy in body mass was anticipated between them since these individuals shared a similar background (i.e., Korean male conscripts in their early 20s during the Korean War).[2]

The results of the one-sample *t*-test showed that the estimated body mass from the 54 individuals (61.3kg) was significantly higher than the reported body mass (56.8kg) by 4.5kg (t=7.383, p<0.001).

In the cylindrical model, the weights of two cylinders with the same volume (i.e., same height and breadth) but different density cannot be identical. Due to a relatively higher percentage of fat in Asian populations, Asian and non-Asian individuals of the same shape (i.e., same stature and body breadth) do not have the same weights. In this case, the non-Asians will be heavier than the Asians, because muscle is denser than fat. In addition, the morphometric method of Ruff *et al.* was mostly based on non-Asian samples with only one Asian population, Japanese, included.[1] Therefore, the morphometric method devised from the non-Asian samples tends to produce overestimated body mass for Asian individuals. In fact, when this method was applied to the referenced Japanese sample using the given stature and bi-iliac breadth data in Ruff *et al.*, the Japanese body mass was also overestimated by 6.2kg and 4.7kg for females and males, respectively.[3]

In applying the Ruff *et al.*'s morphometric method to Asian populations, it is recommended that one be aware of the potential magnitude of bias associated with the method through a validity test and, if available, use an adjustment factor.[1]

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2. Park T., Choung H., Lee M., Chang S. Anthropological studies on the Korean: I. Pro-standard of the length, weight and girth of the chest of recruit. *Med* 1953;1:107-112
3. Ruff C. Morphological adaptation to climate in modern and fossil hominids. *Yearb Phys Anthropol* 1994;37:65-107.

Body Mass Estimation, Morphometric Method, Korean Skeletal Remains

A3 A Two-Pronged Approach to the Identification of Deceased Unidentified Border Crossers in North Carolina: 3D-ID and Geochemical Analysis

Chelsey A. Juarez, PhD, Department of Soc & Anthro NCSU, 1911 Bldg, 10 Current Drive, Campus Box 8107, Raleigh, NC 27695-8107; and Ann H. Ross, PhD*, North Carolina State University, Sociology & Anthropology, Campus Box 8107, Raleigh, NC 27695-8107*

After attending this presentation, attendees will better understand the utility of a two-pronged approach for the identification of deceased undocumented border crossers and, in particular, how this combination of tools is uniquely suited for unidentified persons from Central and South America.

This presentation will impact the forensic science community by providing results from a case representing a growing scenario in the Southeastern United States, the undocumented immigration of Central and South Americans. This presentation will add to research being implemented in forensic anthropology by demonstrating the increased precision for region-of-origin identification when 3D-ID software and geochemical analysis are combined. In addition, this presentation will also discuss the difficulties of dealing with the identification of "non-conventional" undocumented migrant groups (e.g., South Americans rather than Mexicans).

According to the Federal Bureau of Investigation's (FBI's) National Crime Information Center (NCIC) missing person and unidentified person files, as of December 31, 2014, there were approximately 40,000 to 50,000 unidentified dead in the United States. Human remains are thought to go unidentified for many reasons. According to Kimmerle et al., the missing who end up as unidentified are predominantly male, adult, foreign-born individuals, minorities, and at-risk individuals.[1] Undocumented status of many of the unidentified presents a unique challenge in the process of identification as conventional forensic identification tools such as family DNA-reference databases focus on United States citizens with next of kin. Thus, although the ability to solve a cold case is multifactorial, region-of-origin data is a critical component in successfully beginning the identification process.[2] Of the total foreign-born individuals, approximately 56% are from Mexico, 26% are from Central and South America, and as many as 26% of the total population of foreign-born persons are thought to be undocumented. Undocumented persons travel and reside throughout the country and, as a result, states such as North Carolina, Georgia, and Illinois are among the top states with the highest undocumented populations.[3]

The cold case described in this presentation represents one such case from the Wake County Sheriff's Office and Office of the Chief Medical Examiner's in Raleigh, NC. The unidentified remains of a single adult male initially found in 2003 were delivered to the NC State Forensic Anthropological Facility for analysis more than a decade after initial recovery. Biological sex and age estimates indicated the decedent was a male 41.9-53.7 years of age. Craniofacial morphology was characteristic of an individual of Hispanic ancestry. Metric and geometric morphometric ancestry assessment was conducted using both FORDISC® 3.0 and 3D-ID with similar, but critically contrasting, results. Metrically using FORDISC® 3.0, the individual classified as a Hispanic male with posterior (0.476) and typicality (0.655) probabilities. The software 3D-ID, which utilizes coordinate data, classified this individual as South American Male with posterior (0.4683) and typicality (0.4573) probabilities. In order to clarify region of origin, samples of tooth #30, a portion of the right femoral shaft, and a portion of the sternal end of right rib #12 were sampled for carbon, oxygen, and strontium isotopes. Results showed that tissue samples of all types were mechanistically indistinguishable within their categories and not consistent with a North Carolina acclimation, suggesting that the individual had been in North Carolina for less than five years (Bone 87Sr/86Sr ratio: 0.70802; Tooth 87Sr/86Sr ratio: 0.70813). Both samples had a standard error of +/-0.00001 (Femur δ18O value 22.7+/-0.341‰; Rib δ18O value 22.5+/-0.107‰; Enamel δ18O value 22.4+/-0.269‰). Oxygen isotopes in body tissues are obtained primarily from drinking water (~70%). The value of potential drinking water sources from body tissues was estimated to be -11.11‰. Utilizing the region-specific data from the 3D-ID combined with the isotope data, the region of origin for this individual was consistent with Southern Peru, which demonstrated a 87Sr/86Sr range of 0.70728 to 0.70906 and a drinking water range of -11.1 to -12.6.[3,4]

In conclusion, both isotopes and ancestry assessments are more successful at region-of-origin identification when combined. This case study suggests that in the case of undocumented Latinos, the combination of isotopes and 3D-ID may be better able to discern individuals of South and Central American descent and should be considered an important investigative component for forensic anthropologists working with these populations.

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3. Buzon M.R., Conlee C.A., Bowen G.J. Refining oxygen isotope analysis in the Nasca region of Peru: an investigation of water sources and archaeological samples. *Int J Osteoarchaeol* 2011;21:446–455.

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A4 Commingling Among Disinterred Remains of Unknown United States Service Members From the Korean War

Mary S. Megyesi, PhD, JPAC-CIL, 310 Worchester Avenue, Bldg 45, Joint Base Pearl Harbor-Hickam, HI 96853; Nicholas V. Passalacqua, PhD, 1559 Mount Vernon, East Lansing, MI 48823; Popi Chrysostomou, MSc, 6 Kallinou Street, Strovolos, Nicosia 2039, CYPRUS; and Michael R. Dolski, PhD, Defense POW/MIA Accounting Agency, 2211 Ala Wai Boulevard, #1015, Honolulu, HI 96815*

After attending this presentation, attendees will learn the nature and rate of commingling among purported United States service members, buried as unknowns from the Korean War.

This presentation will impact the forensic science community by demonstrating the extent of commingling in historic military cemetery contexts, identify the need for proper analyses with regard to establishing Minimum Numbers of Individuals (MNI), and discuss identification rates from disinterments under similar contexts.

The Defense POW/MIA Accounting Agency (DPAA) is a new Department of Defense (DoD) agency (established January 2015), formed by the merging of several pre-existing DoD organizations including the Joint POW/MIA Accounting Command (JPAC). This newly created agency is responsible for accounting for "persons whose remains have not been recovered from the conflict in which they were lost," specifically in regard to past United States military conflicts (National Defense Authorization Act (NDAA) 2015).

As part of the accounting effort, the DPAA routinely disinters United States service members buried as unknowns from cemetery contexts around the world. The goal of this project is to discuss rates of identification and commingling from disinterred Korean War United States service member caskets. This study examined all Korean War disinterments from the National Memorial Cemetery of the Pacific between 1999-2014. During this period, a total of 91 caskets were disinterred resulting in a total MNI of 108 individuals. In all Korean War disinterments, no commingling was *anticipated*, meaning that at least in theory, each unknown was buried as a single individual and any possible commingling was resolved by analysts prior to the internment of the unknown remains. From the 91 caskets, 16 (18%) were found to have commingling present and represent more than one individual; 1 of these represented an MNI of three, while the other 15 all represented an MNI of two individuals. Of the total 108 disinterred individuals (from the 91 caskets), 55 (51%) have been identified to date, and from the 33 individuals involved in commingled accessions, 10 individuals have been identified.

The vast majority of the commingled remains resulted from duplication of small skeletal elements (e.g., an extra pisiform or phalanx). When the small-element commingling is removed, the commingling rate drops to six caskets (7%), with a total MNI of 96, and the identification rate increases to 58%; however, the small-element commingling introduces a significant issue. The individuals represented by these isolated elements are (with current methods) unresolvable. Including these unresolvable cases in the total MNI results in a decrease of identification rate, while at the same time, we currently lack methods to identify these isolated elements, mainly due to the extremely poor DNA preservation. Accounting for these remains and accurately reflecting the number of individuals that are possible to identify in a commingled casket may become more of an issue as disinterments increase.

The JPAC developed a rigorous process using both historical and scientific assessments of case-related sources to develop lists of potentially associated candidates with each set of unknown remains prior to disinterment. The process was oriented to meet the past United States policy of disinterring only those unknown service members most likely to yield an identification. In April 2015, the DoD announced a new policy regarding the disinterment of unknown United States servicemember remains which lowered the standards required to disinter unknown servicemembers in order to increase the number of disinterments. Following previously established best-practice procedures, the identification success rate for identifiable remains is ~60% to date, with more identifications pending. The Korean War unknowns highlight how a rigorous multidisciplinary assessment of cases prior to disinterment *can* lead to successful and acceptable identification rates. A lowering of the standards to disinter greater numbers of unknowns will very likely have an adverse impact on identification rate, especially considering the current commingling rate present in Korean War unknown caskets. In addition, this policy will also affect WWII disinterments, which are known to have a dramatically higher commingling rate (~76% to date). Since the majority of interred unknowns are associated with WWII, the policy change may ultimately serve to raise disinterment rates, without a subsequent increase in identification rates. The commingling and related identification issues of the Korean War unknowns should inform our disinterment policies and practices, in contrast to escalating exhumations without considering these issues.

The views herein are those of the authors and do not necessarily represent those of the Department of Defense or the United States government.

Disinterment, Commingling, Identification

A5 Death Along the United States-Mexico Border: A Comparative View of Policy and Practice in Arizona and Texas

Kate Spradley, PhD, Texas State University, Dept of Anthropology, 601 University Drive, San Marcos, TX 78666; Robin C. Reineke, PhD, University of Arizona, 1009 E South Campus Drive, Tucson, AZ 85721; Mercedes Doretti, 578A Halsey Street, Ground Floor, Brooklyn, NY 11233; and Bruce E. Anderson, PhD, PCOME, Forensic Science Center, 2825 E District Street, Tucson, AZ 85714*

After attending this presentation, attendees will understand how the differences in medicolegal investigation strategies in two states along the border impact identification efforts of undocumented migrants that die attempting to cross the United States-Mexico border.

This presentation will impact the forensic science community by highlighting alternative strategies for the positive identification of missing and unidentified migrants that die along the United States-Mexico border.

Until recently, the majority of migrant deaths occurred in Arizona despite the fact that the Texas-Mexico border covers 1,254 miles of the 1,900 miles of the entire border; however, in 2012, Texas surpassed Arizona in deaths with the majority occurring in the Rio Grande Valley and more specifically in Brooks County, TX.[1] Unlike most Arizona border counties, most Texas counties with migrant deaths do not keep official statistics or provide systematic medicolegal death investigation for the undocumented migrants that die along the United States side of the border. Therefore, it is difficult to address how many migrants have died in Texas, where they die, and the final disposition of death.

The purpose of this presentation is to explore the differences in medicolegal death investigation efforts for undocumented migrants in Arizona and Texas and the proximate and ultimate factors that contribute to migrant identification. The focus will be on Brooks County, TX, and the Pima County Office of the Medical Examiner in Tucson, AZ, due to their high numbers of migrant fatalities. Comparisons are made between the types of medicolegal systems in each county, a medical examiner system in Pima County that serves three additional counties versus a Justice of the Peace in Brooks County. Each county is reviewed for its identification protocol including number of deaths, identification rate, means of identification, and ultimate disposition of unidentified human remains.

Results indicate that decedents along the border in Arizona are systematically taken to a medical examiner's office, examined by a forensic pathologist or anthropologist, and entered into the National Missing and Unidentified Persons System (NamUs). Identification methods include fingerprints, dental comparison, or DNA and, in some cases, circumstantial evidence. In contrast, Brooks County functions under a Justice of the Peace system. Prior to 2013, when a migrant died in Brooks County, TX, the body was often identified on the spot if Identification (ID) cards were found associated with remains or was most often taken to a funeral home that would attempt identification. If the funeral home was unsuccessful, the remains were buried in a cemetery with no systematic record keeping as to where the remains were buried. Although required by the Texas Criminal Code of Procedures, DNA samples were rarely obtained from the undocumented migrants for submission to the Combined DNA Index System (CODIS), which also requires a NamUs submission.

Between 2001 and 2013, Pima County, AZ, received 2,203 remains of individuals suspected to be undocumented migrants and 1,463 were positively identified, providing an identification rate of 66.4%.[2] In Brooks County, TX, it was published that 129 migrants died in 2013. It is unknown how many migrants were identified and the means of identification. While the final disposition of undocumented migrants is largely known for Brooks County, TX, it is unknown for the rest of the border counties in Texas.

The Pima County Medical Examiner's Office is located in close proximity to the border, employed a forensic anthropologist, and has the ability to use governmental or private laboratories for DNA identification, which contributes to a high identification rate. While three medical examiners exist in close proximity to the Texas border, the lack of funding in Brooks County, prior to 2013, meant that undocumented migrants were not sent to a medical examiner's office or for a forensic anthropological analysis. The situation in Brooks County is similar to most counties along the Texas border. Case studies will be used to illustrate the difficulties of this humanitarian issue along the United States-Mexico border.

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Forensic Anthropology, United States/Mexico, Identification

A6 Sex Determination Using Discriminant Analysis of Upper and Lower Extremity Bones: A New Approach Using the Volume and Surface Area of Digital Models

Dong-Ho Eddie Kim, BSc, 222 Banpo-daero, Seocho-gu, Seoul 137-701, SOUTH KOREA; U-Young Lee, MD, The Catholic Univ of Korea, Dept of Anatomy, Coll of Med, 505, Banpo-dong, Seocho-gu, Seoul 137701, SOUTH KOREA; In-Beom Kim, PhD, The Catholic University of Korea, 222 Banpodaero Seochogoo, Seoul 137701, SOUTH KOREA; and Dai-Soon Kwak, PhD, Catholic Institute for Applied Anatomy, The Catholic University of Korea, 222 Banpodaero Seochogoo, Seoul 137701, SOUTH KOREA*

The goal of this presentation is to propose a new approach for sex determination using the volume and surface area of digital bone models.

This presentation will impact the forensic science community by revealing a new sex determination method using the volume and surface area of bones. Using this method, the ulna has the highest accuracy for sex determination (94%). When utilizing the surface area of multiple bones, the maximum accuracy rate of 99.4% was achieved.

This study analyzed 3D digital models of selected upper and lower limb bones. The volumes and surface areas were calculated and used for sex determination. In addition, discriminant analysis of the volume and surface area of the bones was performed to determine sex.

This study used 110 Computed Tomography (CT) images taken from donated Korean cadavers (55 females and 55 males) to create 3D models of the following upper and lower elements: the clavicle, the scapula, the humerus, the radius, the ulna, the innominate, the femur, the patella, the tibia, the talus, and the calcaneus. The average ages of the female and male samples were 54 years and 55 years, respectively; the average heights were 156cm and 165cm, respectively. Elements showing signs of surgery or deformity were excluded from the study. A medical image-processing program, Mimics® version 16.0, was used to construct the 3D models and determine the surface area and volume of the bones. The 3D models were constructed on the basis of the outer perimeter of the cortical bone and the inner marrow space was not expressed. There were no significant differences between the 3D models and actual bones (p=0.79). Significant sex differences were found in all bones with respect to volume and surface area (p<0.01). The order of volume was the same in female and male (femur > innominate > tibia > humerus > scapula); however, the order of the surface area was different. The largest surface area in males was the femur and in females was the innominate (p<0.01).

The accuracy of sex determination ranged from 72.3%-94.5% in univariate discriminant function analysis for single bones. Regarding the use of volume for sex determination, the radius, humerus, and ulna (in ascending order) were more than 90% accurate; meanwhile, for surface area, the humerus, clavicle, radius, and ulna (in ascending order) were more than 90% accurate. Interestingly, the ulna has the highest accuracy for sex determination (94%).

Discrimination analysis using pairs of bones was over 90% accurate when surface area and volume were used for sex determination. Of 66 combinations, 32 were more than 90% accurate when using volume. Again, the ulna showed the highest accuracy; the combination of ulna with femur, tibia, or fibula showed an accuracy of 95.7%. Regarding surface area, 38 of 66 combinations were more than 90% accurate. The combination of ulna and the clavicle or patella was 95.4% accurate. Thus, paired bones were more accurate than single bones.

When using the surface area of multiple bones, a maximum accuracy of 99.4% was achieved. The equation is as follows: (discriminant equation of surface area; female<0<male)=0.060×clavicle+0.020×scapula+0.045×humerus+(-0.049)×radius+0.093×ulna+(-0.023)×innominate+0.091×patella+(-0.052)×fibula+0.043×talus−11.548. Overall, this study shows that bone volume and surface area can be used for sex determination.

This study revealed that using the surface area for sex determination is more accurate than using volume. Surface area can be calculated regardless of the expression of bone marrow space, broadening its applicability. Study limitations include no broken and/or damaged bones can be used and the samples were limited to Korean individuals. Regardless, the derived sex determination equation using the surface area of various limb bones was 99.4% accurate. Therefore, together with the traditional sex determination methods, this equation can be used for sex determination from available CT, Magnetic Resonance (MR), or 3D scan data. Furthermore, this method is expected to automatically determine sex from existing digitized bone data and make it easier to research other populations.

Sex Determination, Bone Volume, Surface Area

A7 3D Analysis of Computed Tomography (CT) -Derived Lumbar Spine Models for the Estimation of Sex

Robert Foley, MS, Department of Radiology, USF Morsani College of Medicine, 2 Tampa General Circle STC7033, Tampa, FL 33606; Joshua M. Hazelton, BS, Department of Radiology, USF Morsani College of Medicine, 2 Tampa General Circle, STC7033, Tampa, FL 33606; Summer J. Decker, PhD, USF MCOM Dept of Radiology, 2 Tampa General Circle, STC 7033, Tampa, FL 33606; and Jonathan M. Ford, PhD*, Dept of Radiology, 2 Tampa General Circle, STC 7027, Tampa, FL 33606-3571*

After attending this presentation, attendees will more deeply understand the usefulness of 3D CT models of lumbar vertebrae in the estimation of sex from the spine. Attendees will also learn which measurements of the spine are most beneficial and which ones should be used with caution.

This presentation will impact the forensic science community by providing the results of a methodology to assist in forensic analysis, particularly in sex identification. This presentation will enhance existing research of osteological materials by adding data of a living, modern American sample with the spine *in situ*. This will provide insight into the natural spacing and orientation of the spinal components.

Sex identification is a crucial part of the forensic analysis of human remains. While the skull and pelvic bones are often the most ideal structures to use in sex estimation, the condition in which skeletal remains are found is frequently not ideal as bones may be damaged or missing.

Previous studies of the spine and sex estimation have examined the 1st cervical vertebra, 2nd cervical vertebrae, 12th thoracic vertebra, and the 1st lumbar vertebra with varying degrees of success. The hypothesis of this study was that CT-derived 3D models of lumbar vertebrae will be able to capture the unique morphologies used in determining sex in the human body. This study examined all five lumbar vertebrae in order to determine the most reliable and robust method for sex estimation. A series of 140 lumbar vertebrae complexes were acquired from CT and three-dimensionally reconstructed into volumetric models from living patients. The dataset was divided into 70 males and 70 females. Ages ranged from 20 years to 89 years old. Any individuals having additional or missing lumbar vertebrae were excluded from this study. The lumbar vertebrae (L1-L5) and the top of the sacrum were modeled and analyzed using 27 measurements and five aspect ratios for each vertebra. The data were then analyzed using the statistical package SPSS 22. All measurements with a $P<0.05$ were considered to be significant.

Bilateral measurements of the articular and transverse processes, pedicles, facets, and lamina were all compared using a paired t-test and no statistical significance between sides was found. Therefore, any bilateral measurements used in the discriminate function test were limited to the left side. A paired *t*-test was performed comparing males and females for each linear measurement and ratio. Measurements that were determined to be statistically significant were used for further analysis. A stepwise analysis method used these focus measurements to create discriminate equations for L1 though L5 individually.

For L1, five measurements (upper endplate width, upper endplate depth/middle depth ratio, left transverse process length, posterior vertebral height, and anterior vertebral height) predicted sex with 100% accuracy. For L2, five measurements (upper endplate depth, articular process width, spinous process height, lower endplate depth, and upper endplate width) predicted sex with 100% accuracy. For L3, four measurements (transverse process length, anterior vertebral height, spinal canal width, and spinal canal depth) predicted sex with 100% accuracy. For L4, six measurements (transverse process length, posterior vertebral body height/anterior vertebral body height ratio, articular process width, spinal canal width/spinal canal depth ratio, lower endplate width, and spinous process height) predicted sex with 100% accuracy. For L5, three measurements (lower endplate width, transverse process length, and superior articular process height) predicted sex with 100% accuracy.

Human remains in forensic cases are discovered and recovered in scattered, damaged, comingled, or partial states making identification more difficult for those establishing an unknown individual's biological profile. By having a modern living human data sample, investigators can utilize new reference data of any lumbar vertebrae in their quest for a positive identification. The accuracy of the sex estimation found in this study for all lumbar vertebrae reinforces the distinct dimorphism between sexes while also providing forensic practitioners with more options or tools for their analyses.

Lumbar Spine, Sex Estimation, CT

A8 Estimation of Stature From Footprints in a North Indian Population

Kewal Krishan, PhD, Panjab University, Dept of Anthropology, Sector 14, Chandigarh 160 014, INDIA; and Tanuj Kanchan, MD, Dept of Forensic Medicine, Light House Hill Road, Mangalore, Karnataka 575 001, INDIA*

After attending this presentation, attendees will understand the usefulness and methodology of stature estimation, especially from footprints, which will help them study cases pertaining to footprint evidence usually encountered at crime scenes and conduct further research in this area.

This presentation will impact the forensic science community by presenting standards and methodology for stature estimation from various footprint length measurements, which will be helpful in studying footprint evidence encountered at crime scenes.

Forensic podiatry is an up and coming branch of forensic science which deals with the collection, interpretation, and examination of the pedal evidence recovered at crime scenes. The evidence may be in the form of complete and/or partial bare footprints, shoe prints, or a series of footprints that can provide clues to the identity the perpetrator/criminal. The footprint evidence may help to provide identification by the study of individualistic characteristics and features present in the footprints and by providing clues regarding the biological profile of the criminal. The parameters of the biological profile such as stature and sex can be estimated from the size of the footprints. The present study provides correlation of stature with various lengths of the footprint and derives linear and multiple regression models for estimation of stature from these lengths. The sample for the present study is based upon 700 adult participants (500 males, 200 females) with ages ranging from 18 years to 30 years old. The standing footprints were taken from each participant using an inking method. Five footprint length measurements were taken from each subject using the length of the footprint from the anterior-most point of each toe pad to the posterior-most part of the heel impression (i.e., T1, T2, T3, T4, and T5, respectively), according to standard procedures and landmarks. Sex differences in stature and footprint measurements were calculated using a Student's t-test. Pearson's correlation coefficients were calculated between stature and various length measurements of the footprint. Stature was estimated from various length measurements of the footprint using linear and multiple regression analysis.

Mean stature of the study group was 170.30cm and 157.98cm in males and females, respectively. Footprint length at the first toe (T1) was found to be the longest on the left side in males and females. Sex differences in the length measurements of the footprints were statistically significant between males and females for the right and left feet ($p<0.001$). Statistically significant correlation coefficients ($p\leq0.001$) were found for correlation between stature and various footprint length measurements in males, females, and in the pooled sample, except for footprint length of the fourth toe (T4) in females. Thus, stature was found to be positively and strongly correlated to various footprint length measurements in both the sexes. In males, the correlation value (r) ranged from 0.653 and 0.693, while in females it was from 0.558 and 0.665. The correlation coefficient in the pooled sample ranged from 0.672 and 0.698. Thus, the pooled sample showed relatively higher values of correlation coefficients than males and females separately. The linear and multiple regression models were derived for estimation of stature from footprint length measurements in males, females, and the pooled group. Multiple regression models showed a marginally better result, but with a similar trend of accuracy as shown in the males, females, and pooled group in the linear regression analysis. When estimating stature from linear regression models involving all footprint length measurements, the Standard Error of Estimate (SEE) for females (left=3.4cm, right=3.5cm) was lower than that of males (left=4.8cm, right=4.9cm). Observations indicated that female footprints gave a better estimate of stature than did male footprints. Accuracy of stature estimation was marginally better on the left side compared to the right side.

Forensic Podiatry, Stature Estimation, Footprint Length

A9 Sexual Dimorphism in Mandibular Morphology Between Dentate and Edentate Individuals — Implications for Sex Estimation

Heli Maijanen, PhD, University of Oulu, PO Box 1000, Oulu 90014, FINLAND; Beatrix Dudzik, PhD, 250 S Stadium Hall, Knoxville, TN 37996; and Kathleen Hauther, University of Tennessee, 250 S Stadium Hall, Knoxville, TN 37920*

After attending this presentation, attendees will be informed on the morphological changes between dentate and edentate mandibles and their impact on sex estimation.

This presentation will impact the forensic science community by providing quantification of shape variables of the mandible between the sexes in the dentate and edentulous state.

The mandible has traditionally been included in metric and non-metric methods for sex estimation of the skull. The accuracy of methods is debatable, as previous research provides contradictory results depending on which methods and areas of the mandible are used.[1,2] Several studies have found significant changes in mandibular morphology due to tooth loss. Some studies have reported these changes exceed the sexual dimorphism seen in mandibular measurements.[3,4] Thus, the sex estimation of an edentulous mandible may be compromised.

The focus of this study was to identify and quantify sex-related differences in shape and size between dentate and edentate mandibles. Emphasis was placed on regions that have traditionally been used in non-metric sex estimation methods such as gonial angle, chin shape, ramus breadth, and mental eminence. The study evaluated the degree of sexual dimorphism in mandibular morphology and whether this dimorphism is retained with extreme tooth loss.

The sample consisted of 120 individuals, including males, females, dentate, and edentate, from the W.M. Bass Donated Skeletal Collection. Coordinate data were collected using a MicroScribe® G2X digitizer. Twenty-three landmarks were collected that represent morphological areas that are typically used in sex estimation methods. Shape-related differences of dentate and edentulous samples were examined congruently with metric dimensions. Additionally, quantification of shape variables associated with the mental eminence was approximated by the use of a new combination of landmarks, including a novel landmark that makes visualization of shape variation among males and females more feasible when alveolar resorption has occurred.

Preliminary results showed significant differences between males and females in the dentate group in three measurements: bigonial diameter, bicondylar breadth, and mandibular length. All these measurements were greater in males. The differences were smaller in the edentate group. In both males and females, the gonial angle was wider in the edentate group, but no significant sex differences were found in either group. Comparison of shape coordinates of dentate males and females showed significant differences and provided higher accuracy in discriminant analyses than has been reported in previous studies. Differences in the sexes among the edentulous sample were not as significant as with the dentate cohort; however, accuracy estimation percentages were near 80%.

The results confirm the earlier findings that there are changes in mandibular morphology due to tooth loss. These changes seem to diminish the sexual dimorphism seen in dentate individuals in certain areas; however, when shape variables are examined, higher estimation accuracies can be obtained. These results indicate that quantified shape variables should be taken into account if sex estimation from isolated dimensions of an edentate mandible is attempted.

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2. Spradley M.K., Jantz R. Sex estimation in forensic anthropology: skull versus postcranial elements. *J Forensic Sci* 2011;56:289-296.
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Mandibular Morphology, Sex Estimation, Tooth Loss

A10 Evaluating Elongated Pubic Bones as a Potential Sexing Method for Juveniles

Cassie E. Skipper, BS, Texas State University, New Braunfels, TX 78130*

After attending this presentation, attendees will be informed about the issues with current subadult sexing methods and the utility of public bone elongation as a sex indicator in juvenile skeletal remains and its relationship with age.

This presentation will impact the forensic science community by helping to make sexing juveniles a simpler and more realistic task than it currently is, which will aid in identification and the return or curation of unknown remains.

In the field of anthropology, it is important to know the biological profile of individuals in order to understand past population structure, mortality rates, sex-specific burial practices and rituals, and demographics of study samples and populations. This information is critical to increase insight into archaeological populations and to establish standards for research techniques.

Specifically in biological anthropology, having enough information and applicable methods to estimate the biological profile from skeletal remains is imperative for the identification of unknown individuals. Current literature on juvenile sexual dimorphism is lacking, and existing publications on juvenile sexing methods have been known to result in low correct classification rates.[1] Furthermore, Holcomb and Konigsberg attest to a wide overlap between the two sexes and question the accuracy of current sexing methods in juveniles.[2] These issues lead to the hindrance of juvenile sex estimation analyses and the identification process. Cognizance of sexual dimorphism patterns and human variation within and between populations is important in order to produce a stronger academic and research foundation for the field and their resulting real world applications.

Bass noted the elongation of the pubic bone as being a female trait.[3] This trait is often informally recognized by biological anthropologists, but it is seldom officially identified and used in non-metric trait studies. As of yet, pubic elongation and radiographic images of juvenile pubic bones have not been utilized as a potential method to quantify sexually dimorphic traits in subadults.

The present research includes a preliminary evaluation of juvenile pubic bones for sexual dimorphism. Radiographs of 20 juveniles from 9 years to 12 years of age were selected at random from the Pediatric Radiology Interactive Atlas (Patricia®) database.[4] Each individual was scored three times for presence/absence of elongated pubic bones. Three rounds of scoring were used to ensure reliability of this method and to serve as intra-observer error for future research in this study.

Chi-square goodness of fit and Cramer's V statistics were employed to evaluate the significance of the results. Sex (X^2=10.769, p-value=0.001; Cramer's V=0.734, p-value=0.001) was found to have a stronger correlation with the presence of elongated pubic bones than age (X^2=6.848, p-value=0.039; Cramer's V=0.569, p-value=0.039). Females under the age of ten years used in this research were incorrectly classified as male based on their lack of pubic elongation.

Preliminary findings indicate that this method accurately classifies males aged 9 years to 12 years old and females aged 10 years to 12 years old. Other methods for sexing juvenile skeletal remains are often unreliable, inconsistent, and/or require extensive amounts of data and funding. The method proposed here is less expensive and more efficient than established methods and can have a profound effect on the identification rates of unknown juveniles.

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1. Vlak D., Roksandic M., Schillaci M. Greater sciatic notch as a sex indicator in juveniles. *Am J Phys Anthropol* 2008;137(8):309-315.
2. Holcomb S.M.C., Konigsberg L.W. Statistical study of sexual dimorphism in the human fetal sciatic notch. *Am J Phys Anthropol* 1995;97(2):113-125.
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Juvenile Skeletons, Sex Estimation, Pubic Bone

A11 Age Estimation Using the Sternal End of the Clavicle: A Test of the Falys and Prangle Archaeological Method for Forensic Application

Meghan Price, Boston University School of Medicine, 72 E Concord Street, Boston, MA 02134; James Pokines, PhD, Boston University School of Medicine, Dept of Anatomy & Neurobiology, 72 E Concord Street, L1004, Boston, MA 02118; and Jonathan D. Bethard, PhD, Boston University School of Medicine, Dept of Anatomy & Neurobiology, 72 E Concord Street, L1004, Boston, MA 02118*

After attending this presentation, attendees will understand the reliability of a new age estimation method designed to increase the accuracy of aging individuals who were more than 40 years of age.

This presentation will impact the forensic science community by emphasizing the need for and application of age estimation methods that accurately age older individuals. This presentation discusses a new method for estimating age from the sternal end of the clavicle, as described in Falys and Prangle and tests this age estimation method on a modern American sample.[1]

Age estimation is a critical component of the biological profile in forensic and bioarchaeological contexts. The majority of these methods are most accurate for individuals of younger age cohorts, typically those less than 40 years of age. Skeletal degeneration can vary greatly between individuals, making age estimation less accurate for adult individuals. While there are some methods that attempt to age older individuals accurately and precisely, more research must be conducted to expand the range of methods available. Falys and Prangle developed a method for estimating age in individuals who were more than 40 years of age using three characteristics of the sternal end of the clavicle: (1) surface topography; (2) porosity; and, (3) osteophyte formation.*1*

In order to test their method, a sample of 1,510 individuals of known sex and age, ranging from 20 years to 101 years of age (males: *n*=1,112, mean=50.57, Standard Deviation (SD)=18.015; females: *n*=398, mean=53.065, SD=20.358), were drawn from the McCormick Collection and the William M. Bass Donated Skeletal Collection at the University of Tennessee.

The two estimation methods proposed in Falys and Prangle, regression equation and composite score, were tested to see how well they performed when applied to the collected data.[1] When applied to the collected data, the regression equation produced age estimations within the 95% confidence interval in 47.6% of the male sample and 57.4% of the female sample. Composite scores were calculated and compared to the corresponding age ranges provided by Falys and Prangle.[1] The composite scores of the male sample estimated the age of an individual more accurately than the composite scores of the female sample (male=65.9%; female=58.8%). The lowest estimation accuracy for both males and females was between 70 years to 79 years of age (male=46.0%; female=51.4%). From 80 years to 89 years of age, the accuracy increased for males (76.4%) and females (69.4%).

The sample also included individuals less than 40 years of age in order to test the applicability of this method. Multiple regression equations were generated: (1) individuals more than 20 years of age; (2) individuals less than 30 years of age; and, (3) individuals more than 40 years of age. The results from the multiple regression analyses show comparable Pearson's coefficients for the above-mentioned equations (r=0.690, r=0.632, r=0.611, respectively).

Spearman's rank correlation coefficients indicated a correlation significant at the 0.01 level for all three components individually, as well as the composite score. Of the three components, surface topography was most strongly correlated with age for both males (r=0.643) and females (r=0.590). Unlike the findings of Falys and Prangle, porosity was found to be the least correlated with age for both males (r=0.474) and females (r=0.514).[1] In addition, when broken down into ten-year intervals (40-49, 50-59, etc.), the correlation coefficients increased with advancing age. This suggests that the method becomes more accurate as the age of an individual increases.

The findings in the present study indicate that the sternal end of the clavicle has potential for use in age estimation in older individuals. Although the present study produced lower correlation coefficients than proposed by the original study in 2014, the results suggest this method has the potential to provide accurate and precise age ranges for older individuals.

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Age-at-Death Estimation, Clavicle, Forensic Anthropology

A12 Accuracy of Dental Age in Non-Adults: A Comparison of Two Methods for Age Estimation Using Radiographs of Developing Teeth

Sierra Santana, BA, Boston University School of Medicine, 72 E Concord Street, Boston, MA 02118; Jonathan D. Bethard, PhD, Boston University School of Medicine, Dept of Anatomy & Neurobiology, 72 E Concord Street, L1004, Boston, MA 02118; and Tara L. Moore, PhD, 700 Albany Street, W701, Boston, MA 02118*

After attending this presentation, attendees will better understand the principles of estimating age in non-adults, current methods that utilize radiographs for age estimation, and their application to the forensic and legal communities.

This presentation will impact the forensic science community by expanding knowledge of the current methods for estimating age in non-adults using dental radiographs by providing a comparison of two dental age estimation methods outlined in Cameriere et al. and AlQahtani et al.[1,2]

Estimating age at death of an individual is an important factor within several scientific fields, with direct application to forensic, archaeological, and legal settings. The goal of this presentation is to provide an assessment of the accuracy and applicability of two recently published methods for age estimation in non-adults, Cameriere's European formula for age estimation and AlQahtani's London Atlas on a multi-ethnic American population.[1,2] These two methods are of particular interest because initial research demonstrates that these methods may produce a more accurate and precise age estimate than the methods currently used in the field of forensic anthropology.[3]

This study utilized dental radiographs drawn from the Maxwell Museum of Anthropology's Orthodontics Case File System at the University of New Mexico. The sample consisted of 363 panoramic radiographs from individuals aged 7 years to 17 years old (mean age=11.9 years) with each individual having been identified as having affiliation with one of the following ethnicities: American Indian, Hispanic, or White/European American. A Dental Age (DA) estimation was performed for every radiograph twice, once using the method outlined by Cameriere and once using the London Atlas. The Chronological Age (CA) of each individual is calculated as the difference between the date of birth provided in the dental record and the date on which the radiograph was taken. For each method, accuracy and bias were determined. The accuracy of DA estimation is defined as how closely CA can be predicted. Bias is defined as the mean difference between DA and CA and can be either a positive or negative number and is used to determine if a method overestimates or underestimates an individual's age. Furthermore, categories relating to ethnicity, sex, and age were applied to the assessment of accuracy and bias in order to compare the two methodological approaches.

The age of each individual was calculated as the difference between the date of birth provided in the dental record and the date on which the radiograph was taken. Preliminary statistics demonstrate a significant positive correlation ($p<0.01$) between DA and CA for both the London Atlas ($r=.87$) and Cameriere's ($r=.72$) method.

To test intra-observer reproducibility, a random sample of 40 panoramic radiographs was re-examined after an interval of two weeks and tested using Pearson's correlation coefficient. Results indicate that there were no statistically significant intra-observer differences between the paired sets of measurements carried out on the re-examined panoramic radiographs for either method.

Estimated age was closer to CA using the London Atlas than Cameriere's method. The London Atlas underestimated age by approximately 0.12 years (Standard Deviation (SD)=2.3) for males and 0.21 years (SD=2.8) for females. Cameriere's method underestimated CA by approximately 1.43 years (SD=1.64) for males, 1.76 years (SD=1.64) for females. In regard to true age, the mean CA was 11.99 years for girls, with Cameriere's method producing a mean DA of 10.23 years and the London Atlas producing a mean DA of 11.76. For boys, the mean CA was 11.95 years with the mean DA being 10.40 years for Cameriere's method and 10.23 years for the London Atlas. Both methods underestimated CA for both sexes. Furthermore, in regard to Cameriere's method, it is suggested that a new regression formula specific to an American population should be created.

Reference(s):

1. Cameriere R., Ferrante L., Cingolani M. Age estimation in children by measurement of open apices in teeth. *Int J Legal Med* 2006;120:49-52.
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Age Estimation, Non-Adult, Dental Radiograph

A13 A Test of Cervical Vertebral Ring Union for Age-at-Death Estimation Using the Albert-Sherwood Method

A. Midori Albert, PhD, University of NC Wilmington, Dept of Anthropology, 601 S College Road, Wilmington, NC 28403-5907; and Kate D. Sherwood, 10401 Litzsinger Road, St. Louis, MO 63131*

After attending this presentation, attendees will gain a more in-depth understanding of the applicability of the Albert-Sherwood vertebral ring epiphyseal union method for estimating the age of unknown skeletons. This presentation seeks to explain how the method may be applied, reports on its accuracy, for cervical vertebrae, and suggests contexts for its considered use in age estimation.

This presentation will impact the forensic science community by demonstrating how a specific skeletal age estimation method may be used and by explaining the meaningfulness and utility of the results that may be obtained. Information presented here may serve forensic anthropology practitioners as well as researchers interested in the further study of this approach to age estimation.

The purpose of this study was to test the accuracy of estimating age at death by examining the progress of cervical vertebral maturation using epiphyses of the centra that were in poor condition to simulate real-world forensic contexts. Further, another goal of this study was to test the accuracy of estimating age at death on a sample derived from a population differing from the reference/guideline sample. Ages were estimated using cervical vertebral data originally collected from a population differing from the test sample since in the practice of forensic anthropology there are often issues with applying guidelines developed on one sample but used for another — inasmuch as different samples may be affected by cross-population variability and or secular changes in skeletal growth, development, and degeneration.

The test sample for this study was derived from the Lisbon Collection housed at the Museu Nacional de História Natural, Museu Bocage in Lisbon, Portugal. It contained vertebrae that were deteriorated, damaged, and/or characterized by fragile epiphyseal rings. The test sample comprised 20 individuals (7 female, 12 male, and 1 individual of indeterminate sex), ranging in age at death from 10 years to 30 years old; however, the sex and age at death were unknown at the time the aging method test was conducted (i.e., this was a blind study). Due to the discovery of 1 of the 20 individuals not having any cervical vertebrae available for analysis, the final test sample included 19 individuals.

Guidelines used to estimate the ages at death for the test sample were developed from vertebral ring union data obtained from the Hamann-Todd Osteological Collection housed at the Cleveland Museum of Natural History in Ohio. The guideline sample comprised 100 individuals between the ages of 10 years to 30 years old at death: 55 African Americans (34 females and 21 males) and 45 European Americans (19 females and 26 males). The sample was selected by sorting the collection according to sex and age at death, and individuals were randomly selected for each age in years to ensure continuous representation of the maturation process, as much as possible. The progress of cervical vertebral ring union was documented in five stages (i.e., the Albert-Sherwood method, revised from the Albert-Maples method): (1) Stage 0 was no union; (2) Stage 1 was beginning and active union; (3) Stage 2 was complete union with no remodeling of the gaps between the epiphysis and centrum; (4) Stage 3 was complete union with some (incomplete) remodeling of the gaps; and, (5) Stage 4 was complete union with complete remodeling (note: this stage may not be attained in all vertebrae as occasionally a "scar" remains). The guidelines for age estimation, based on this five-stage method, include key ages at which the stages of vertebral ring union are first attained (Stages 0-4), at what ages they are sustained (Stages 0-4), and/or the ages at which they are surpassed (Stages 0-3; Stage 4 is the final stage and if/when attained, it persists).

Ages were estimated by comparing the test sample stages of union for each individual (age and sex unknown during this part of the study) with the guidelines for the earliest and latest ages for various stages of union. Since sex differences were found to exist when the guidelines were developed, not knowing the sex during the test of the method yielded wider intervals of estimated ages. Results indicated that age estimation intervals were correctly assessed for 16 out of 19 individuals (84%), spanning ages 10 years to 30 years old. For the three individuals incorrectly assessed, they were estimated to be no older than 10 years of age; however, the actual ages were 13 (one male) and 14 years (two males). This finding suggested that ring epiphyses may have broken off, giving the appearance of bare centra for which no epiphyseal union had begun, but in reality may have been in progress or it could be that maturation may simply begin later in the population from which the test sample was derived. Further details of the findings and their implications for the utility of this method are discussed. Overall, this method is promising for use in the field, particularly in conjunction with other skeletal age indicators and when sex can be adequately assessed.

Vertebral Maturation, Age Estimation, Epiphyseal Union

A14 Age Estimation Using Osteophytic Activity on the Lumbar Vertebrae and Partial Least Squares Regression

Jacob Griffin, BS, 16665 Danville Road, Danville, IA 52623; and Stephen D. Ousley, PhD, Dept of Anthropology/Archaeology, Mercyhurst University, 501 E 38th Street, Erie, PA 16546*

After attending this presentation, attendees will better understand how to estimate the age at death of adult individuals using osteophyte development on the vertebral centra and the utility of the prediction intervals presented in the methodology.

This presentation will impact the forensic science community by presenting a method that can be used to estimate the age at time of death of adult individuals when only the lumbar vertebrae are present. This presentation also shows the need to continue further studies into the utility of osteophytes for biological profile estimation, for they are an area with very little previous research.

Historically, there have been four areas that are typically used for adult age estimation.[1] These areas include the auricular surface, sternal rib ends, pubic symphysis, and cranial sutures. Other areas of the skeleton have been investigated with the same scrutiny. One area that has received very little attention is the osteophytic activity on the vertebral column. These osteophytes are frequently used to obtain general age estimations when employing the *Gestalt* method of getting a feel for the age of the decedent based upon the morphological appearance of several areas; however, there has been little work performed to develop quantitative or qualitative adult age estimation methods using osteophytosis since the work of Stewart.[2,3]

Snodgrass continued research into osteophyte development on the vertebral centra using revisions made to the Stewart scoring system.[3,4] Snodgrass used inappropriate statistics for ordinal data and his method is difficult to apply due to a lack of description and illustration.[4] It is difficult to apply the developed scoring system to osteophytic development, especially for those with a limited understanding of the developmental patterns of osteophytes on the vertebral centra.

A total of 203 White males and females of known ancestry, sex, and age were sampled from the Hamann-Todd Collection at the Cleveland Museum of Natural History. The superior and inferior rims of each lumbar vertebra were scored using the Snodgrass five-stage, ordinal scoring system.

Polychoric correlation matrixes showed there is a high degree of correlation between the superior and inferior rims of all five vertebrae. The highest correlation found between two vertebrae was 0.92 and the lowest correlation was 0.69. The polychoric correlation also found that the variables are all highly correlated and, thus, multicollinearity is an issue. Analysis of Covariance (ANCOVA) showed that the differences between the mean scores for males and females across age were not statistically significant with an F-value of 0.429. Partial least squares regression was chosen for age estimation over other methods because it eliminates the problem of multicollinearity. The partial least squares model was then used to create 95% prediction intervals using R software.[5]

The 95% prediction intervals reported in this presentation are quite large with the interval being approximately 50 years. Many forensic anthropologists argue that this method is of little use in narrowing down the missing persons list due to its large age intervals; however, the lower bounds of these intervals do provide important cut-off points. For example, if an individual scores a three on all of the vertebral rims, the observer can conclude, with 95% confidence, that he or she is more than 46 years of age. This finding can eliminate a large portion of possible identifications and would be useful when combined with other age estimation methods. Osteophytosis can be used as a method for age estimation, but it does require further research and validation. Revisions need to be made to the scoring system developed by Snodgrass.

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Age Estimation, Osteophytes, Vertebrae

A15 The First Thoracic Vertebral Centrum as an Adult Age Estimation Site

Kristina B. Altes, MA, CA Pound Human Identification Laboratory, 2033 Mowry Road, Rm G-17, Cancer/Genetics Research Complex, U of FL, Gainesville, FL 32610*

After attending this presentation, attendees will be aware of a new adult age estimation site that may supplement current sites, such as the pubic symphysis and sternal rib ends.

This presentation will impact the forensic science community by providing a new adult age estimation site when others are unavailable in skeletal remains.

The thoracic vertebrae have received relatively little attention as age estimation sites compared to the pelvic joints, the ribs, and the cranial sutures. Albert and Maples established juvenile and young adult age estimates based on annular epiphysis fusion, while Stewart and Snodgrass found correlations between osteophyte development and adult age; however, none of these studies focused on specific vertebrae, and none have attempted to provide a "gestalt" description of the aging thoracic centrum.[1-3]

Photographs of the inferior centra of 168 undamaged first thoracic vertebrae from the Robert J. Terry Anatomical Skeletal Collection at the Smithsonian National Museum of Natural History were examined. The sample consisted of 48 White males, 44 Black males, 37 White females, and 39 Black females. The inferior aspect of first thoracic vertebra centra were assigned to one of five phases defined by degree of epiphyseal ring obliteration, texture, symmetry, and edge irregularity. Inter-observer error was assessed by using 14 first thoracic vertebrae from the C.A. Pound Human Identification Laboratory (CAPHIL) archives. The same vertebrae were scored one week later to assess intra-observer error. Similar tests of error were performed using the Brooks and Suchey method on 14 left pubes, also from the CAPHIL archives, for comparison.[4]

Age correlated with phase relatively well within the pooled sample (R^2=0.25) and even better when sex was considered separately (R^2=0.27 for males and 0.30 for females). An Analysis of Variance (ANOVA) test demonstrated that phases differ significantly from one another in mean age (p=2.39x10^{-10}), while a Games-Howell post-hoc test demonstrated that Phases 1 and 2 differed significantly from Phases 4 and 5; Phase 3 from Phase 5; Phase 4 from Phases 1, 2, and 5; and Phase 5 from all other phases. In addition, inter- and intra-observer errors were smaller when using this method than when utilizing the Suchey-Brooks pubic symphysis method: inter-observer kappa for the thoracic vertebrae was 0.51 and 0.70, indicating moderate and substantial agreement between observers, respectively. In contrast, inter-observer kappa was 0.30 for each pair of inter-observer comparisons for the pubic symphyses, indicating fair agreement. Intra-observer kappa was 1.0 for the thoracic vertebrae and 0.83 for the pubic symphyses, indicating perfect and almost perfect agreement, respectively.

These results suggest the first thoracic vertebral centrum may be a useful adult age estimation tool, in addition to more commonly utilized joint surfaces. Future research will develop this method more thoroughly on a more modern population.

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Forensic Anthropology, Age Estimation, Vertebrae

A16 Improved Adult Age Estimation Using New Skeletal Traits and Transition Analysis

George R. Milner, PhD, Department of Anthropology, 409 Carpenter Bldg, Pennsylvania State University, University Park, PA 16802; Jesper L. Boldsen, PhD, ADBOU, Institute of Forensic Medicine, Lucernemarken 20, 5260 Odense S, DENMARK; Stephen D. Ousley, PhD, Dept of Anthropology/Archaeology, Mercyhurst University, 501 E 38th Street, Erie, PA 16546; Svenja Weise, PhD, Institute of Forensic Medicine, University of Southern Denmark, Odense, DENMARK; Sara M. Getz, MS, Penn State University, Dept of Anthropology, 409 Carpenter Bldg, University Park, PA 16802; and Peter Tarp, MS, Institute of Forensic Medicine, University of Southern Denmark, Odense, DENMARK*

After attending this presentation, attendees will better understand new directions in age estimation for adult skeletons, as well as preliminary results from a National Institute of Justice (NIJ) grant that involves an integrated approach featuring new skeletal age markers, analytical methods, and computer program development.

This presentation will impact the forensic science community by highlighting ongoing progress made in improving the accuracy and precision of adult skeletal age estimation methods, focusing on the use of better-defined skeletal features, statistically sound methods, and software to aid in recording and analyzing age-informative skeletal markers.

Accurate age-at-death estimates are essential in forensic identifications from skeletal remains. Yet despite a century of research, accurate and precise age estimates are beyond what standard methods employed by forensic anthropologists can provide, especially for individuals older than approximately 45 years of age. Estimates of age have traditionally incorporated information from the cranium, pubic symphysis, and sacroiliac joint. Unfortunately, these areas only yield information about the first few decades of adulthood and are often so variable among individuals that they fail to yield satisfactory estimates. Age estimates spanning decades contribute little or nothing to narrowing possible identifications. Only recently have age estimation methods incorporated advanced statistical methods, such as transition analysis.[1]

Standard age estimation procedures suffer from poor reliability (low agreement between observers in feature scores), low precision (large age estimation intervals, frequently "eyeballed"), and poor accuracy (agreement between estimated and actual age), problems that have been recognized in bioarchaeology for more than three decades.[2] Commonly used procedures also have open-ended terminal intervals (e.g., 50+ years), rendering them worthless for identifying elderly people. These problems have made adult age estimation one of the biggest challenges for forensic anthropologists, especially in the post-*Daubert* era.

Fortunately, the situation is not as bleak as might be imagined.[3] A recently awarded NIJ grant builds on promising pilot studies to develop a procedure that incorporates three interrelated and essential features: (1) the definition of new skeletal traits and the collection of data on their ages of transition; (2) the incorporation of mathematical approaches to properly analyze age-related information; and, (3) the development of a computer program that will enable practitioners to make effective use of the new skeletal information and analytical procedures. In short, substantive improvement requires a thorough rethinking of what is examined, how skeletal traits are used, and the production of a computationally heavy but user-friendly computer program. To have a major impact on forensic investigations, all three tasks must be completed simultaneously. This initiative is part of a broader trend in osteological research that owes inspiration to *Daubert* guidelines for reliable and valid age estimates, coupled with greater accuracy, explicit prediction intervals, and greater applicability to diverse populations.[4]

Preliminary results derived from more than 400 individuals from the Universities of Tennessee and Pretoria show that the new approach produces better results than commonly used procedures. Many parts of the skeleton, not just a few parts, are informative about age. Traits have been investigated in all major long bones, ribs, vertebrae, pelvis, skull, and select bones of the hands and feet. Valuable age-informative transitions have been derived for traits in many of these areas, including the humerus, femur, sacrum, and other areas on the innominate. When ages of transition from several features are combined, they show how skeletal traits that are rarely, if ever, used in existing methods measurably enhance the accuracy and precision of age estimates throughout adulthood. These preliminary results, based on only part of the eventual total sample, were compared to the limited information that can be gleaned from the pubic symphysis and sacroiliac joint, the parts of the skeleton most commonly used for age estimation. The new features perform better for age estimation than those areas.

The main preliminary results show that age-informative markers are distributed throughout the skeleton, they can be analyzed to provide information about age throughout the adult lifespan, and the predicted ages are more accurate, precise, and replicable.

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Age Estimation, Transition Analysis, Skeleton

A17 Effects of Scavenging Birds and Insects on Decomposition Time of Pig Carcasses at the Rice Creek Field Station

Brianna L. Robinson, 112 Ketchum Road, Conklin, NY 13748; and Kathleen A.S. Blake, PhD*, State University of NY at Oswego, Dept of Anthropology, Mahar 441, Oswego, NY 13126*

After attending this presentation, attendees will better understand the role of avian scavenging during the decomposition process and how presence and timing of avian species differs between seasons in upstate New York.

This presentation will impact the forensic science community by providing an understanding of how avian species, habitat, and temperature ranges in the Northeast have different impacts on scavenging and carrion preservation levels. This presentation will add knowledge to an area of little research pertaining to avian scavengers and their impact on decomposition particularly in upstate New York.

A few studies have focused on avian scavenging or briefly discussed their contribution to the decomposition process; however, this subject has been severely understudied in the temperate Northeast.[1-4] Compared to mammals, avian carnivores are known to be more adapted with their abilities to scavenge for carrion.[5] The effect of this region's climate and geography both play a role in the type of scavengers seen.

For this research, four separate 10'-diameter plots of 2"x4" mesh fencing were placed throughout the grounds at two different environmental locations in Rice Creek Field Station at the State University of New York at Oswego, NY. The control plot fencing was covered with bird netting to allow for natural decomposition without the effect of any scavengers. One pig (*Sus scrofa domesticus*) carcass was placed in each plot during the autumn of 2014 and the spring of 2015 (n=8). The two environments were open grassland and woodlands, both with an accompanying control. The autumn experiment ran from October 1 until December 1 and the spring experiment was from March 2 until late May. During decomposition, wildlife cameras were set out and checked weekly during midday hours.

The fall experiment pigs were predated on by a single avian species, turkey vultures. Maggots dominated the percentage of insect activity and had the most effect on decomposition. Vultures visited both the open and wooded experimental sites for only week two of the project. The average feeding time for vultures was 28.7 minutes over the course of five days. Observations found all pigs decayed by 80% within four weeks of placement. Insects primarily affected the rate of decomposition, with scavenger activity minimal. Insect activity was focused on the head and shifted downward as decomposition progressed.

The spring experiment results showed a 100% species variety increase and 193% increase of scavenging individuals. Red-tailed hawks and vultures were both seen within a few days of placement. Vultures continued to visit the forest site past week six of decomposition. The average feeding time for avian species in the spring was 30.8 minutes over the course of 19 days. The damage and scavenging done by avian species was higher by 800% in the spring but still did not heavily affect decomposition. Decay was delayed substantially, with no signs present within the first four weeks due to colder weather conditions in the spring. All pigs that were predated upon were scavenged mostly in three specific locations; these included the anal region, head, and torso.

This study's findings differ from others, particularly a study in Texas, which found that black vultures and turkey vultures completely skeletonized the pig in 3 to 27 hours of arrival.[6] A case in southern Illinois, on the other hand, observed a delay in the time of first arrival of the vultures but still found complete skeletonization within two months.[2] This study found that scavenging was minimal with no complete skeletonization from avian species in either season.

In conclusion, this study provides evidence that while avian species may be present during the decomposition process, they may not heavily impact the rate of decay. Seasonal changes in the environment affected the abundance and variety of avian species seen at each site as well as insect activity present. Furthermore, data gained from this research can be used to illuminate the differences from other regions as well as caution that scavenging data from these areas cannot be applied to the Northeast.

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Scavenging, Decomposition, Taphonomy

A18 The Mummy in the Microwave: The Efficacy of the Microwave Method for the Maceration of Desiccated Tissue

Christiane Baigent, MSc, Metropolitan State University Dept Sociology/Anthr, PO Box 173362, Campus Box 28, Denver, CO 80217-3362; and Gary T. Scott, MA*, Metropolitan State University of Denver, Dept of Anthropology and Sociology, 1201 5th Street, Campus Box 28, Denver, CO 80204*

After attending this presentation, attendees will better understand decomposition as an important variable when selecting a maceration method and specifically, how the efficacy of the microwave technique is interrupted by desiccated tissue.

This presentation will impact the forensic science community by presenting observations made in a controlled laboratory environment on a subject for which little published data exists. Further, desiccation is considered on a molecular level and introduced as a new variable for consideration when selecting a method for tissue removal.

The maceration of human remains in the forensic anthropology laboratory has received variable attention in the literature. For logical reasons, an early emphasis was placed on the preservation of bone composition and gross morphology. More recently, the focus has shifted to the effect of various maceration techniques on nuclear DNA preservation and the preservation of microstructures associated with traumatic lesions.[1-3] Among the suite of methods tested in these studies is the microwave technique, in which skeletal elements are placed in a microwave-safe dish, loosely covered with a lid or plastic wrap, and microwaved on high for one-minute intervals until all soft tissue "easily slip[s] from the bones."[1] The use of both *Sus scrofa* and human bone and associated soft tissue is reported in the literature with consistent positive results. Absent from these studies is a discussion regarding decomposition as a variable and the potential for its many stages to differentially affect the efficacy of a maceration method. Because it has been lauded for its ease of use, and tested positively in the preservation of both DNA and the micro-morphology of osseous lesions, the microwave method was selected for use in a recent analysis conducted by the Metropolitan State University of Denver Human Identification Laboratory (MSUD-HIL). This presentation reports the results of the use of the microwave method in a case in which overlying soft and connective tissue structures were present in various stages of desiccation.

The skeletal elements associated with the left shoulder girdle, arm, and hand of an adult male were recovered from a high-altitude outdoor site north of Denver, CO. Desiccated dermal and connective tissue was present and mummification was observed in the hands, characterized by the preservation of all dermal layers and integumentary accessories (nails, eponychium, and hair). Prior to processing these remains, the technique was performed on eight *Sus scrofa* ribs; the results of preliminary processing were consistent with published data. The human remains were then processed in the MSUD-HIL. Bones were sequentially placed in a glass dish containing 2mm of water and covered with plastic wrap, then heated using a microwave (2.2 cu. ft., 1,250W) set on high for one-minute intervals. The process was numerically scored following Steadman et al. to quantify odor, soft tissue texture, ease of tissue removal, and bone quality.[1] The resultant bone quality scores were consistent with published results, but odor, tissue texture, and ease of tissue removal (and subsequent processing time) varied greatly from published scores. Additionally, scores between skeletal elements varied greatly, with the clavicle demonstrating the greatest ease of tissue removal and the scapula and carpals presenting the most difficulty.

The results suggest the structural changes associated with dehydrated cartilage and connective tissue has a substantial effect on the maceration of human remains and should be considered prior to engaging in tissue removal. Zhu and Fang report that the nanostructure of dehydrated cartilage is characterized by inhomogeneous fibril D-periodic spacing (decreased tissue organization), increased fibril diameter (greater bulk and density), and an increase in surface rugosity (with attendant changes in surface area expected).[4] These structural changes proved to be reversible during laboratory rehydration, making it is reasonable to suggest that the added process of rehydration affects maceration time as the distance from the denaturation threshold is increased. The increased processing time may have bearing on extant concerns surrounding DNA recovery and analysis and the preservation of trauma morphology, indicating that desiccated tissue should be regarded as a significant variable in the maceration process. Therefore, further testing is recommended to address tissue removal throughout the continuum of decomposition. While these observations do not outweigh the empirically tested benefits of the microwave method, they do suggest that more careful consideration is warranted.

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Microwave Maceration, Desiccation, Mummification

A19 The Effect of Plastic Tarps on the Rate of Human Decomposition During the Spring/Summer in Central Texas

Chloe P. McDaneld, 125 Amberwood Cove, Kyle, TX 78640; and Daniel J. Wescott, PhD, Texas State University, Dept of Anthropology, 601 University Drive, San Marcos, TX 78666-4684*

After attending this presentation, attendees will gain a better understanding of decomposition rates of human remains wrapped in tarps and whether they are different than unwrapped human remains using Total Body Scores (TBS) and Accumulated Degree Days (ADD).

This presentation will impact the forensic science community by adding to the research on the effects of decomposition when the body is covered or wrapped in a tarp. It will also contribute to time-since-death estimation.

Forensic case reports cite that bodies are commonly covered or wrapped in man-made materials for disposal and concealment.[1-3] Therefore, knowing whether there are differences in the rate of decomposition between wrapped and unwrapped bodies is important for forensic scientists conducting estimations of time since death. While several studies have been conducted on the effects of decomposition when the body is covered or wrapped in materials such as clothing, blankets, and plastic tarps, most of these studies have examined a variety of coverings simultaneously with relatively small sample sizes.[4-9] Therefore, the purpose of this study was to conduct a controlled investigation of the effect of plastic tarps on the rate and pattern of decomposition in Central Texas using a relatively large sample size. Unlike previous studies, this study utilized only one type of covering, the sample size was larger than previously examined, and environmental conditions and dates of death were known.

Human remains covered or wrapped in a tarp provide the perfect environment for decomposition since the tarp may maintain moisture and temperature while providing insects and bacteria protection from the sun and rain. Therefore, it was hypothesized that the plastic tarp would aid in decomposition in two ways: (1) by increasing the activity of necrophagous insects, which prefer a warm, shaded, and outdoor environment; and, (2) by increasing putrefaction caused by bacteria that require an aqueous medium.[10-12] The increased activity of insects and bacteria would therefore likely increase the rate of decomposition or, in other words, require fewer ADD to reach each stage of decomposition.

The study sample consisted of 20 bodies wrapped in plastic tarps and a matched control sample of unwrapped bodies, both placed on the ground surface in a tree-covered area of the Forensic Anthropology Research Facility at Texas State University. The TBS was compared between the wrapped and control bodies at 500 ADD and 1,000 ADD.[13] *T*-tests were used to test for statistical significance.

Statistical analyses showed that tarps primarily have an effect on the rate of decomposition after 500 ADD. There were no significant differences in TBS between the human remains wrapped in plastic tarps and the unwrapped remains at 500 ADD (p-value=.118036). While the rate of decomposition was not significant, it was observed that for the bodies wrapped in tarps, the head and neck region decomposed faster (higher TBS) compared to the control group; however, at 1,000 ADD, there was a significant difference in TBS between human remains wrapped in plastic tarps and the unwrapped remains (p-value=.0456).

The results show that during the early decomposition period, plastic tarps do not have a significant effect on the rate of decomposition, but the tarp may affect the observed pattern of decomposition. As the decomposition process continues, insect activity associated with the unwrapped bodies decreased and the bodies began to desiccate; however, the consistent warm and shaded environment in the tarps allowed for continued insect activity and slowed desiccation. As a result, bodies wrapped in plastic tarps had a greater TBS after 500 ADD than unwrapped bodies. These results suggest that ADD calculations based on TBS may underestimate the time since death for a body wrapped in a tarp if the individual has been deceased for more than 500 ADD.

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Human Decomposition, TBS, Tarps

A20 Effect of Body Size on the Rate of Outdoor Human Soft Tissue Decomposition

Lindsey G. Roberts, MA, 912 E Cindy Street, Carbondale, IL 62901; and Jessica R. Spencer, MA, 424 S Front Street, Cobden, IL 62920-2415*

After attending this presentation, attendees will understand the effects of body size on the progression of outdoor human soft tissue decomposition.

This presentation will impact the forensic science community by contributing to a greater understanding of a key variable affecting decomposition and the estimation of Postmortem Interval (PMI).

This research examined differences in the decomposition rate of human subjects with respect to body size. Previous studies have primarily focused on animal models, and yielded conflicting results concerning the impacts of body size on the rate of decomposition, with some suggesting no effect, while others found subject mass to be a key factor in which smaller subjects progressed more rapidly through decomposition than larger subjects, and yet another found mass to differentially affect decomposition depending on the stage of decomposition.[1-6] Due to the liquefaction of adipose tissue and the results of previous studies, this research hypothesized that larger subjects would decompose more rapidly during early decomposition (6.0≤Total Body Score(TBS)<19.0) but less rapidly during advanced decomposition (19.0≤TBS<27.0).[6-7]

Eleven human subjects donated to the Complex for Forensic Anthropology Research (CFAR) at Southern Illinois University (SIU) were placed unclothed, supine, directly on the ground surface within the complex between December 7, 2012, and March 3, 2015. Subjects were placed between 2m-25m apart, resulting in almost identical research environments, and protected from avian and mammalian scavengers by chain-link cages. Subject samples included eight males and three females between the ages of 49 years and 95 years with the following body weights (kg): 73, 77, 84, 104, 109, 112, 113, 127, 136, 136, and 159. After deposition, TBS, photographs, and written qualitative descriptions concerning subject appearance and insect activity were collected daily. Accumulated Degree Days (ADD) were used to assess the thermal energy required for each subject to reach several TBS landmarks: early decomposition (TBS≥6.0); midpoint between early and advanced decomposition (TBS≥12.5); advanced decomposition (TBS≥19.0); halfway through advanced decomposition (TBS≥23.0); and skeletonization (TBS≥27.0).[7]

Preliminary statistical testing showed no significant positive or negative correlation between body weight and ADD at any TBS landmark. At TBS≥6.0, body weight accounted for 2.4% of the variation in ADD (r=0.155, p=0.65, n=11). At TBS≥12.5 (halfway through early decomposition), body weight explained 9.8% of the variation in ADD (r=0.296, p=0.377, n=11). At advanced decomposition (TBS≥19.0), 9.3% of variation in ADD was explained by body weight (r=0.305, p=0.36, n=11). Midway through advanced decomposition (TBS≥23.0), 4.1% of variation was explained by body weight (r=-0.202, p=0.55, n=11). At advanced decomposition (TBS≥27.0), 26.6% of the variation in ADD was explained by body weight (r=-0.516, p=0.29, n=6). Results suggested there is only a minor influence of body weight on the rate of outdoor decomposition in southern Illinois. Although not statistically significant, the correlation between body weight and ADD was positive until midway through advanced decomposition when this relationship became negative: larger-sized individuals required fewer ADD than smaller subjects to reach TBS≥23.0 and TBS≥27.0. The study's hypothesis was not supported by the results: there was no statistically significant correlation between decomposition rate and body size during any stage of decomposition.

In conclusion, results of this preliminary study suggest body weight is not a significant factor in driving human decomposition nor should it significantly impact PMI estimation. Additionally, it should be noted 2.4%-26.6% of variation in decomposition rate was explained by body weight depending on the stage of decomposition. Further research is necessary and ongoing.

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A21 A Methodology in Differentiating Between Knives From Cut Marks on Bone

Melodi Ghui, Liverpool John Moores University, Byrom Street, Liverpool, AE L3 3AF, UNITED KINGDOM; Constantine Eliopoulos, PhD, Liverpool John Moores Univ, School of Nat Science & Psych, James Parsons Bldg, Byrom Street, Liverpool L3 3AF, UNITED KINGDOM; and Matteo Borrini, PhD, Liverpool John Moores University, RCEAP-School of Natural Science & Psych, Byrom Street, Liverpool L3 3AF, UNITED KINGDOM*

The goal of this presentation is to propose a flowchart as an additional tool to enhance the assessment of cut marks on bones.

This presentation will impact the forensic science community by offering a new implement to recognize characteristics of cut marks and provide an effective method to correctly identify the type of knife used.

Many studies in sharp force trauma discuss knife cut mark analysis in the context of dismemberment in murder cases; however, blunt force trauma and sharp force wounds are the most common injuries in crime, especially in homicides.[1] Furthermore, sharp force trauma has been debated as being the leading cause of murder in the United Kingdom.[2] The research in lesion identification on bones has been successful, particularly in determining the type of blade used (serrated or non-serrated); however, there has been no uniformity in the characteristics used to identify the weapon's type.[3,4]

This study was conducted with the goal of creating a standard method for knife identification based on specific characteristics detectable in the marks left on bone tissues. This study chose, as a starting model, the characteristics used for sword and saw cut marks analysis, adapting them to the study of knife injuries and specific characteristics (e.g., grooves).[5,6]

In this study, a total of 150 cut marks were made on domestic pig (*Sus scrofa*) rib bones. These bones were macerated to ensure complete removal of tissue before beginning the experiment. Three different categories of blade were used to inflict cuts on the surface of the bones: non-serrated; micro-serrated (eight Teeth Per Inch with, average distance between teeth: 3mm); and, macro-serrated (five Teeth Per Inch with, average distance between teeth: 4.9mm). During the experiment, the knife was moved one time forward and backward to simulate a stabbing action.

After microscopic analysis (10x7-10x45), χ^2 tests of independence were performed for all characteristics to determine the relation between trait and type of knife. According to the probability of correct identification of knife type by each characteristic, a flowchart was developed. The features were structured from the distinction between serrated and non-serrated, and then between micro- and macro-serrated blades. Four characteristics were chosen for the differentiation between serrated and non-serrated knifes: grooves along the kerf wall; flaking; kerf shape; and general aspect. An additional feature, the presence of shards, is used for the separation between micro- and macro-serrated blades.

A blind test on an additional 100 cut marks was performed. The accuracy of the identification with the support of the flowchart is very high (95%) in the diagnosis between non-serrated and serrated knifes; however, there is a difference when the kind of serration is analyzed as well (0.7%). This suggests that the flowchart needs further improvement in this area, with additional features for the distinction between micro- and macro-serrated blades.

To test how intuitive the use of the proposed flowchart is and the characteristics used, two groups of forensic anthropology students (five undergraduates and five postgraduates) were tested. None of them had received training on cut marks, but they had different degrees of experience in human anatomy and osteology. All undergraduates had significant differences when compared to more experienced individuals, while the results of the postgraduate students closer to the expected values. This result demonstrates that, even if an in-depth knowledge and training in osteology is a prerequisite, the proposed flowchart is a useful tool that has the potential to increase the reliability of knife cut mark analysis. In addition, its use appears to be intuitive and supports the possibility of introducing this method as a teaching tool in graduate programs.

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Cut Marks, Forensics, Flowchart

A22 Traumatic and Congenital Anomalies of the Atlas: A Forensic Identification Case Report

Yann Delannoy, MD, Forensic Taphonomy Unit, Rue André Verhaeghe, Lille 59000, FRANCE; Thomas Colard, DDS, PhD, Institut de Médecine Légale, Place de Verdun, Lille, Nord 59045, FRANCE; Tania Delabarde, PhD, Institut Médico-légal, 2 place Mazas, Paris 75012, FRANCE; Jocelyn Pollard, MD, Place De Verdun, Lille 59045, FRANCE; Valéry C. Hedouin, MD, PhD, Iml-chu Lille, Rue Andre Verraeghe, Lille 59000, FRANCE; and Didier Gosset, MD, PhD, Institut de Medecine Legale, Faculte de Medecine, Lille 59045, FRANCE*

After attending this presentation, attendees will recognize which traumatic and congenital abnormalities of the craniovertebral junction can be used to identify skeletal remains.

This presentation will impact the forensic science community by providing information on the various existing traumatic and congenital anomalies of the cervical atlas. These rare abnormalities provide important data that could lead to a positive identification, such as in this case report.[1]

A partially skeletonized and unidentified body was discovered during the winter in northern France, hidden by vegetation and partially submerged in a swampy area. The identification card found in his clothes corresponded to a 48-year-old man. The victim had an old psychiatric illness and escaped from the hospital during the previous summer. Despite searches conducted by the police, he was never found.

Anthropological analysis revealed that the victim was an adult male, 170cm to 180cm in height, and 36 years to 54 years of age, which was compatible with the presumed identity. A callus was observed on the left clavicle and the examination of the cervical spine showed an old fracture of the atlas with two disjointed zones: one on the anterior arch and another on the posterior arch of the vertebra. Bone margins showed osseous changes associated with skeletal healing processes and remodeling (a Computed Tomography (CT) scan was performed in order to document this injury). The medical records of the victim mentioned an old fracture of the distal end of the left clavicle in 2006, compatible with the postmortem findings, without any other traumatic injury. Given the specific psychiatric history (schizophrenia and chronic alcoholism), several CT brain scans were performed during his hospitalizations and their analyses confirmed the atlas condition: a bursting fracture called the Jefferson fracture. This old fracture was never treated surgically and was never reported in the medical record (there was no sign of neck injury in the decedent's medical past). These bifocal fractures are rare and should not be confused with congenital abnormalities of the craniovertebral junction.

Unlike other cervical vertebrae that develop embryologically from three ossification nuclei (one in the vertebral body and one in each lateral mass), the atlas grows from two lateral ossification centers.[2] Around the seventh week of intrauterine life, ossification begins and extends dorsally. During the second year of life, a separate ossification center appears from the posterior tubercule of the atlas and these posterior arches fuse between three and four years of age. For the anterior arch, one or more ossification centers could appear during the first year of life, but it is possible that no ossification center arises; in that case, the anterior arch is formed from the lateral masses. The fusion is complete between six and eight years of age.[3] Because arches fuse anteriorly and posteriorly progressively, hypoplasia or aplasia might occur on the arches, as well as fusion anomalies (rachischisis or clefts). A rachischisis is possible for the anterior or posterior arches, and the "split atlas" is a fusion anomaly of both anterior and posterior arches. It is therefore important to recognize a split atlas as these osseous gaps may mimic a Jefferson fracture.

The Jefferson fracture results from an axial compressive force applied to the vertex with the neck held rigidly erect.[3] These arch fractures usually occur near the lateral masses (as in this case) and are difficult to observe on conventional radiographs. A CT scan is therefore essential for diagnosis.

Numerous congenital anomalies of the atlas vertebra exist. They must be known to the pathologists and anthropologists to differentiate them from fractures, because many fractures in this area are treated conservatively: Jefferson fractures are typically treated by a hard collar immobilization, provided that the transverse atlantal ligament is considered intact.[2-4] Furthermore, their rarity in the population can be a key element in identifying skeletal remains.

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Positive Identification, Cervical Atlas, Congenital

A23 Comparison Between Peri-Mortem Blunt Force Trauma Identified in Bone During an Autopsy and During an Anthropological Examination of 21 Skeletonized Remains Several Years After Death

Luisa Marinho, MSc, Simon Fraser University, Dept of Archaeology, 8888 University Drive, Burnaby, BC V5A1S6, CANADA; and Hugo Cardoso, PhD, Simon Fraser University, Dept of Archaeology, 8888 University Drive, Burnaby, BC V5A 1S6, CANADA*

After attending this presentation, attendees will understand how an anthropological examination of skeletonized human remains can differ from an autopsy of a fresh cadaver in the identification of peri-mortem blunt force trauma to bone and what the specific circumstances of each examination are that can potentially explain any discrepancies.

This presentation will impact the forensic science community by increasing awareness about the limitations of both the autopsy and the anthropological examination, particularly in cases in which blunt force trauma is involved. Data generated from this comparison will emphasize the importance of a detailed investigation of skeletal trauma during an autopsy, as well as the influence of taphonomic factors that affect the preservation of skeletal material, as these have a negative impact on a thorough identification and subsequent interpretation of trauma mechanisms.

In this study, the number and location of peri-mortem fractures identified during the autopsy of 21 fresh cadavers were compared to the number and location of peri-mortem fractures identified during an anthropological examination of the same individuals several decades after death. These 21 individuals were selected from the identified skeletal reference collection housed at the National Museum of Natural History and Science (NMNHS, *n*=20), in Lisbon, Portugal, and the Collection of Identified Skeletons curated at the Life Sciences Department, University of Coimbra (CEI-UC, *n*=1), Portugal. These individuals are of known cause of death and were selected on the basis of a reported violent death associated with a blunt force mechanism. The autopsy reports generated for these individuals were examined at the archives at the National Institute of Legal Medicine and Forensic Sciences, in the Southern (Lisbon) and Centre (Coimbra) Delegations. Violent deaths due to other trauma mechanisms, such as gunshot wounds or sharp trauma, were not considered. Only 1 of the 21 cases analyzed had perfect correspondence between the number and location of peri-mortem fractures identified during the anthropological examination and the autopsy. A few cases had minor inconsistencies, while the majority of the individuals showed several more significant discrepancies.

This research explores the reasons that may explain these discrepancies and highlights the fact that fractures resulting from a blunt force mechanism are particularly susceptible to misidentification. If, on one hand, the identification of peri-mortem fractures during an anthropological examination is heavily influenced by taphonomic processes, rendering them undetectable at worst or their interpretation dubious at best, then, on the other hand, fractures that do not contribute to the cause of death can be missed during the autopsy or only vaguely reported. This study also draws attention to the value of identified skeletal collections where cause and manner of death are known, as they are invaluable sources of information for the study of skeletal trauma.

Peri-Mortem Fractures, Autopsy, Taphonomy

A24 Reassessing Blunt Force Trauma to True Rib Heads Utilizing Tension-Compression Theory

Kelsey A. Carpenter, BS, Howell, MI 48843; Kena Ihle, BA*, 440 W 9th, #11, Erie, PA 16502; and Steven A. Symes, PhD, Mercyhurst University, 501 E 38th, Erie, PA 16546*

After attending this presentation, attendees will better understand the influence that biomechanical factors have on fracture propagation of blunt force trauma rib heads. This will be achieved by the explanation and analysis of tension and compression.

This presentation will impact the forensic science community by providing preliminary answers to the unanswered question of how rib heads react during blunt force impact. This investigative study will help both forensic anthropologists and pathologists begin to better interpret rib fractures when presented with an unknown scenario.

Rib trauma is commonly used as a last interpretative resort in cases where repetitive trauma is present. This may largely be due to the complicated nature of rib anatomy and physiology complicating individual rib trauma analyses. There appears to be a lack of understanding of ribs on a biomechanical level when mapping and interpreting incomplete and buckle fractures from males and females of various ages.[1] An additional rib study reported that age and sex have little effect on blunt force fractures, suggesting that the study of geometric properties of ribs is imperative for further understanding of blunt force rib fractures.[2]

Introductory research investigating rib cortical area, section modulus, linear structural stiffness, and skeletal robusticity found the expected, in that robust bones are more resistant to bending and therefore more structurally sound when force is applied.[3] While the aforementioned study has taken steps in understanding the biomechanical properties of rib fractures, rib head, neck, and tubercle biomechanical studies have yet to be examined specifically and in terms of tension and compression. It is felt that the complex anatomical positioning of rib heads, accompanied by their robust nature, must reveal a significant diagnostic biomechanical pattern of blunt force fracture propagation in the thoracic region.

In this pilot study, ten rib head, neck, and tubercle fractures from five individuals were used. Only true thoracic ribs (ribs four through eight) from males and females with reported blunt force trauma to the chest were used for this study. The sample consists entirely of individuals examined by a member of this study. Scenarios for age at death and cause of death of each individual were previously recorded. Each fracture was analyzed macroscopically using a Leica® MZ16A microscope with 3.5x to 40x magnification capability. Tension and compression were identified on all fracture surfaces during analysis to assess the forces and behavior of the bone as it fractured.

Analysis of the ten ribs heads revealed consistent anatomical fracture patterns. Of the rib heads analyzed, 90% exhibited fractures originating medial to the rib angle, continuing around the tubercle, and propagating into the rib head. The remaining 10% of rib heads analyzed displayed only a hairline fracture between the head articular facet and tubercle, suggesting the blunt force impact was not great enough to fully radiate the fracture. The preliminary research has discovered that rib head fractures avoid more dense cortical bone (stress resistors), like that found at the tubercle, in all scenarios. The behavior of the fractures observed in this study is a result of the anatomical structure of the rib, including the immovable attachments to the vertebrae body and transverse process. The surrounding musculoskeletal and cartilaginous tissues also absorb the impact energy.

In conclusion, this research delves into the examination of blunt force rib head, neck, and tubercle fractures in an effort to better understand how ribs, as individual bones and as a unit, react to force. The ultimate goal of this study is to introduce effective means of interpreting blunt force trauma of the ribs by diagnosing tension and compression in each fracture. The increased accuracy of trauma analysis through biomechanically recognized bone bending can only contribute to an accurate understanding of bone bending and failure.

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Biomechanics, Blunt Force, Ribs

A25 Comparability of Macroscopic, Microscopic, and Radiologically Defined Pediatric Antemortem Healing Stages

Cliff Boyd, PhD, Radford University, Dept of Anthropological Science, Radford, VA 24142; Donna C. Boyd, PhD, Radford University, Forensic Science Institute, PO Box 6939, Radford, VA 24142; Sharon Roller, 56 Harrison Avenue, Waldwick, NJ 07463; and David Foley, BS, Radford University, Dept of Anthropological Sciences, Radford, VA 24142*

The goal of this presentation is to compare macroscopically, microscopically, and radiologically based standards for assessing and interpreting pediatric antemortem fracture healing.

This presentation will impact the forensic science community by providing forensic anthropologists and pathologists with an understanding of the accuracy and potential problems associated with antemortem pediatric fracture interpretation based on different media and contexts. This research will demonstrate that there is considerable variation in observation of macroscopically, microscopically, and radiographically defined healing stages. Recognition of this variation will ultimately aid in more accurate identification of pediatric antemortem fractures in a forensic setting and lead to more precise determinations of time since injury for these fractures.

Recognition and dating of antemortem pediatric fractures in a medical examiner setting may first occur with the use of radiography, followed, in some cases, by macroscopic (gross), microscopic, and histological observation. Radiographic standards for antemortem pediatric bone healing have been derived from observation of immobilized fractures (of a usually accidental origin) in a clinical non-forensic context restricted in temporal extent. In contrast, macroscopic antemortem fractures observed in a forensic context may not have undergone immobilization and are often attributable to a non-accidental etiology. Temporal range of these fractures (e.g., time since injury) may be much broader.

In this study, more than 700 digital macroscopic, microscopic, and radiographic (both digital and analog) images depicting antemortem healing from 55 fractures originating from seven known forensic pediatric death (child abuse) cases are evaluated for the presence of diagnostic characteristics typically observed in bone healing. These characteristics include, but are not limited to, the presence of localized inflammation, rounding of fracture margins, subperiosteal new bone formation, organization of callus, hard callus formation, presence of distinct fracture lines, and resorption of fracture lines. These bone healing signatures are evaluated in relation to established macroscopic, microscopic, and radiographic standards for antemortem fracture healing and the total number of features observed in each medium compared across groups.[1-3]

Results of this study indicate limitations in radiographic-based identification and interpretation of antemortem fractures. This is especially characteristic of the diagnosis of very recent fractures, as well as aged fractures in late stages of remodeling. Identification of subperiosteal new bone formation, distinct fracture lines (particularly in the rib cage), and metaphyseal fractures are often occult in radiographic images. The highest percentage of identified indicators of the healing process through radiography occurred within the middle reparative stages due to the presence of callus formation. While macroscopic imaging allows greater observation of antemortem healing characteristics compared to radiography (particularly in the early and later stages), microscopic imaging reveals an increased number of clear healing features and holds the greatest promise for dating of these fractures.

These results indicate the following: (1) use of established standards for pediatric antemortem fracture identification, interpretation, and dating are heavily dependent upon the medium used (gross observation, microscopy, radiology) to define the stages; (2) specific standards should be developed for the appropriate medium used to assess and date antemortem fractures; (3) reliance on radiography for identification and interpretation of antemortem pediatric fractures is problematic and those standards developed in clinical settings may not be comparable to forensic ones; and, (4) microscopic imaging of the antemortem healing process is strongly recommended in cases of suspected child abuse.

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Pediatric, Antemortem, Healing

A26 A New Statistical Approach to Morphological Sexing of South African Remains

Samuel R. Rennie, BSc, Liverpool John Moores University, Rm 439a James Parsons Bldg, Byrom Street, Liverpool L3 3AF, UNITED KINGDOM; Margaret Clegg, PhD, University College London, Gower Street, London WC1E 6BT, UNITED KINGDOM; and Silvia Gonzalez, PhD, Liverpool John Moores University, Byrom Street, Liverpool, Merseyside L3 3AF, UNITED KINGDOM*

After attending this presentation, attendees will better understand the Summary Sex methodology and how it can be used to assess human remains regardless of population affinity.

This presentation will impact the forensic science community by demonstrating how using the population-specific equations and a universal South African equation makes it possible to estimate sex with a high degree of accuracy.

Anthropologists agree that the most sexually dimorphic element in the human skeleton is the pelvis. This can be explained by the strong selection pressure for bipedality and childbirth; however, when sexing the pelvis, different morphological features can display conflicting results when analyzed separately, so an overall assessment of multiple indicators is generally best. Sadly, in many forensic and archaeological cases, complete remains are rare, and forensic specialists normally have to attempt estimating a biological profile using fragmented remains.

Summary Sex is a multivariate approach to sex estimation that uses a Principal Components Analysis (PCA). This is achieved by analyzing ordinal data collected from up to nine morphological features on the human pelvis. Each of the morphological traits are scored between -2 (hyper-feminine) and +2 (hyper-masculine) with 0 being an ambiguous score. First, only complete remains are analyzed, and a linear equation is created from the first Principal Component (PC). Second, if a specimen is missing any data, the median value for that specimen is calculated and replaces all missing scores. For example, the specimen has five of the nine morphological features present which have the scores of -2, -1, -2, 0, -1, then the remaining four missing scores would have the value of -1. The median was chosen to replace missing values as it follows the amount of sexual dimorphism that is seen in the pelvis and doesn't create a "muddying" effect when plotted.

Two South African samples were created using the modern-day skeletal collections of the Pretoria Bone Collection (housed at the University of Pretoria) and the Raymond Dart Collection (housed at the University of the Witwatersrand). As these are cadaver-based collections, age, sex, and ancestry are known for each individual. South African Whites (*N*=193) and Blacks (*N*=204) were analyzed separately to create specific equations for each group. From this, comparisons were made between the two equations and were tested against each other to assess the possibility of different percentage accuracies. A second analysis was performed which pooled the two samples together to form an overall South African sample (*N*=397) to create a new equation which was compared against the equations created from the population-specific equations.

The Summary Sex equation for the specific groups resulted in 94.82% accuracy for South African Whites and 89.95% for South African Blacks. When comparing results, the South African White equation on the South African Black data resulted in 89.48% accuracy and the South African Black equation on South African White data was 94.82%. When pooling both groups, correct classification of sex was 92.70%. When this overall equation was then applied to only Black or White South Africans, it resulted in accuracies of 89.95% and 94.82%, respectively.

What this methodology shows is that, unlike Discriminant Function Analysis where knowledge is needed *a priori*, a PCA approach has the ability to classify males and females with high accuracy. Also, Summary Sex allows a researcher to observe the range of sexual dimorphism present within a given population. Furthermore, Summary Sex shows the capability of analyzing specimens that are fragmented and still retain a high percentage of accuracy.

Principal Components Analysis, Pelvis, Forensic Anthropology

A27 Estimating Ancestry in South Africa: A Comparison of Geometric Morphometrics and Traditional Craniometrics

Rebecca King, MS, 7710-T Cherry Park Drive, #383, Houston, TX 77095; Jonathan D. Bethard, PhD, Boston University School of Medicine, Dept of Anatomy & Neurobiology, 72 E Concord Street, L1004, Boston, MA 02118; and Donald F. Siwek, PhD, Dept Anatomy and Neurobiology, Program in Forensic Anthropology, 72 E Concord Street, Boston, MA 02118*

After attending this presentation, attendees will better understand: (1) the accuracy of two ancestry estimation software programs, FORDISC® 3.1. and 3D-ID, using discriminant function analysis in an international population; (2) which of these programs performed more accurately in the South African Black and White populations respectively; and, (3) suggestions for the improvement of these programs to make them more readily useful in contexts outside of the United States.

This presentation will impact the forensic science community by revealing accurate methods of estimating ancestry in South African individuals and distinguishing between Black and White individuals in the population. This presentation will encourage a broader scope in the utilization of ancestry estimation software programs created in the United States as forensic contexts abroad become more relevant.

In ancestry estimation of South African individuals, non-metric morphological trait assessment has not proven useful and previous results using FORDISC® leave room for improvement.[1,2] Results, when compared against the Forensic Databank (FDB) of FORDISC® 3.0 and a custom-made South African Database (SADB), both linger below the universally accepted accuracy of 75% for use in a forensic context.[2]

The accuracy rates of software programs FORDISC® 3.1 and 3D-ID were compared for ancestry estimation based on cranial data of Black and White South Africans using discriminant function analysis. Cranial landmarks were digitized using a MicroScribe® G2 for geometric morphometric analysis in 3D-ID, and traditional craniometric measurements for use in FORDISC® were calculated from these points using the data collection software 3Skull. Data was collected from a total of 385 individuals (186 Black and 199 White crania) from the Pretoria Bone Collection, University of Pretoria, South Africa. Overall accuracy rates of 75.6% using FORDISC® 3.1 and 63.1% using 3D-ID were obtained for Black and White South Africans. An assessment of intra-observer error was performed using intra-class correlation coefficients and all data showed high correlation between separate measurements of the same individual. Previous studies of inter-observer error in the use of a MicroScribe® to obtain cranial data showed agreement between Type I and Type II landmarks, with some dissent when collecting Type III landmarks.[3]

Higher accuracy rates were obtained when sex of the individual was already known or sex estimates made by the programs were disregarded. Incorrect estimates were more often due to misclassifications of sex rather than ancestry, reflecting the decreased amount of sexual dimorphism in South African populations when compared against American populations, discussed previously.[2] Black South Africans were more often classified correctly in FORDISC® 3.1, and White South Africans were more often classified correctly in 3D-ID, showing opposing biases in the two programs.

Low sample size in comparative databases and broad ancestral differences between South Africans and the proxy populations used, which included American, European, and African, likely explain the low accuracy rates. The accuracy rates obtained in FORDISC® 3.1 are slightly above 75%, making the program acceptable for use in a forensic context to estimate the ancestry of Black and White individuals in South Africa. 3D-ID has performed poorly in this population, though in some cases the program estimated ancestry correctly when FORDISC® 3.1 estimated the ancestry of the same individual incorrectly. Though FORSDISC® performed more accurately than 3D-ID, the use of both programs in conjunction can help South African anthropologists in estimating ancestry and ensuring correct classifications.

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Ancestry, FORDISC®, 3D-ID

A28 Spatial Analysis on a Global Scale: Cranial Non-Metric Trait Variability

Joseph T. Hefner, PhD, Michigan State University, Department of Anthropology, 355 Baker Hall, East Lansing, MI 48824; and Caitlin C.M. Vogelsberg, MS, Michigan State University, Dept of Anthropology, 354 Baker Hall, East Lansing, MI 48824*

After attending this presentation, attendees will understand some principles of cranial non-metric trait analysis with geospatial tools, the necessary elements for the application of geospatial analysis in biodistance studies, and an example of the practical application of geospatial analysis.

This presentation will impact the forensic science community by quantifying global variation in cranial non-metric traits through novel geospatial tools that permit the visualization of qualitative variables.

Cranial non-metric trait (*sensu stricto*) variation in ancestry estimation has been documented by multiple researchers, but the global distribution of that variation using geospatial analyses and large samples has not been explored. Using a dataset of nearly 8,000 individuals, this study explored global spatial variation and the underlying patterns of trait distributions in an effort to fine-tune the analysis of cranial non-metric traits in the estimation of ancestry. Prior to analysis, a selection of Ossenberg's database (n=4,579) was appended to include centroid data (decimal degrees) for each individual from each World Region, each Major Region (by country), and each Country Region (by specific region). To explore how cranial non-metric traits vary around the world and to determine which traits are more useful for population estimations, Ossenberg's dataset was subjected to Principal Components Analysis (PCA) and Principal Coordinates Analysis (PCO) using the generalized inverse of each variable. These data were analyzed within a geospatial framework modified for biodistance analysis.

Experimental variograms explore the relationship between a biological distance measure and the physical (spatial) distance between individuals while providing empirical information regarding the magnitude, extent, and pattern of spatial correlation. The pattern of the variogram suggests the level of spatial autocorrelation. For example, an undulating variogram indicates a correlation between physical distance and biological distance. Following variogram analysis, this study used the calculated spatial correlation to interpolate values between individuals and the empty space between them to construct a smooth plot from contour data using a kriging method. Kriging is a regression method used to estimate unsampled values using a weighted average of known values from nearby individuals. Unlike ad hoc methods, kriging is weighted using the specific underlying pattern of spatial correlation and variation derived from the variogram analysis. The result is a map highlighting the relationship between the individuals or populations. Levels of global spatial autocorrelation were measured using Moran's I, which tests the probability that the amount of spatial clustering present is not due to random chance. Moran's I ranges from 1 to -1, where I >1 indicates a statistically significant positive autocorrelation and I <1 a negative autocorrelation. Additionally, a minimally variable z-score is computed following random permutation calculations (N_p=199 for this analysis). This score also indicates statistical significance for positive autocorrelation when z >1.96.

Following PCA, the factor loadings were examined to determine trait clustering. The first PC explained nearly 40% of the sample variation. The highest loadings for this PC all centered on the highly canalized structures of the basicranium, particularly in the basiocciptal region (e.g., the highest loadings for the first PC were transverse fissue of basiocciput, odonto-occipital articulation, precondylar tubercles). In direct contrast, the second PC, which accounted for an additional 12% of the variation, predominately loaded cranial non-metric traits of the mandible, demonstrating a shift in importance to the lower face. The results of this spatial analysis may explain this shift. These results indicate positive spatial autocorrelation for each of the first four PCs. The Moran's I for the four principal components were, respectively, I=0.07163 (z=~45), I=0.122443 (z=~81), I=0.294553 (z=~190), and I=0.06075 (z=~40). These values indicate that although the second PC explained less of the variation in the sample, it has more spatial dependence (clustering) than the first, while the third PC has the highest level of clustering of these four. Visualization of these clusters sheds light on their patterns of variation and how they shift across space.

This research supports the applicability of cranial non-metric traits in the estimation of ancestry, beyond merely supplemental notes collected during more "trusted" metric or macromorphoscopic analyses. Geographic spatial clustering of grouped cranial traits is evident and, therefore, these traits should be investigated when attempting to assess the ancestry of unknown human remains.

Forensic Sciences, Forensic Anthropology, Geostatistics

A29 Examining Inter-Observer Reliability of Metric and Morphoscopic Characteristics of the Mandible

Jennifer F. Byrnes, PhD, University of Hawaii - West O'ahu, 91-1001 Farrington Highway, Kapolei, HI 96707; Michael W. Kenyhercz, PhD, University of Tennessee, 250 S Stadium Hall, Knoxville, TN 37996; Samantha C. Torres, BA, University of Hawai'i - West O'ahu, Div of Social Sciences, 91-1001 Farrington Highway, Kapolei, HI 96707; and Gregory E. Berg, PhD, DPAA Identification Laboratory, 310 Worchester Avenue, Joint Base Pearl Harbor-Hickam, HI 96853-5530*

After attending this presentation, attendees will understand the reliability of metric and morphoscopic characteristics of the mandible for sex and ancestry estimation.

This presentation will impact the forensic science community by providing inter-observer tests of reliability using observers of varied experience levels for sex and ancestry estimation methods focused on the mandible.

To date, there has been no large study of the reliability of metric and morphoscopic traits of the mandible. Berg provided the forensic anthropology community with a method for the estimation of sex and ancestry using 6 morphoscopic and 11 metric characteristics of the mandible from various world populations.[1,2] A combination of morphoscopic and metric variables were shown to discriminate sex and ancestry best. Further, intra-observer error was shown to be low.

An inter-observer agreement study of the mandibular variables was conducted by four researchers with varied experience using the standard descriptions of metrics, as well as new metric descriptions and scoring criteria described by Berg.[1,2] The standard metric variables included GNI, HML, TML, GOG, CDL, WRB, XRH, MLT, MAN, and two new measurements, mandibular body breadth at the M2/M3 junction (TML23) and dental arcade width at the third molar (XDA). The morphoscopic traits included Chin Shape (CS), Lower Border of the Mandible (LBM), Ascending Ramus Shape (ARS), Gonial Angle Flare (GAF), Mandibular Torus (MT), and the Posterior Ramus Edge Inversion (PREI). The sample data were derived from the William M. Bass Donated Skeletal Collection at the University of Tennessee, Knoxville. In total, this study examined 183 mandibles (white females=66; White males=96; Black females=2; and Black males=19) from known individuals and an additional 189 mandibles (White females=91; White males=88; Black females=4; Black males=6) were also examined.

To test the agreement among observers, the Intra-Class Coefficient (ICC) was used. The ICC quantified the proportion of variance that was ascribed to observations. For reliability, both complete agreement and consistency were evaluated using a two-way, random model ICC with a 95% confidence interval. Additionally, for the metric variables, the Technical Error of Measurement (TEM) was calculated. Both ICC and TEM were calculated with four observers for one test and three experienced observers in a second test in order to examine the effect of experience on agreement.

The ICC for the morphological variables for four observers ranged from 0.37 (PREI) to 0.75 (MT). The ICC values for the three experienced observers ranged from 0.43 (LBM) to 0.74 (MT). The majority of morphoscopic ICC values were between 0.67 and 0.74 (three observers). Each of the ICC values was significant at $p < 0.001$. The ICC values for the metric variables for four observers ranged from 0.73 (TML23) to 0.98 (CDL). Experienced observers ranged from 0.80 (TML23) to 0.99 (WRB). The majority of metric ICC values were between 0.91 and 0.99, and all were significant at $p < 0.001$. The TEM of the metric variables ranged from 1.16mm or 1.00% TEM (CDL) to 1.62mm or 10.53% TEM (TML). The TEM for MAN was 2.19 degrees or 1.74% TEM.

The results show that each of the variables has significant correlation among observers, though the metric variables were more accurately replicated than the morphological traits. Experience plays a role for scoring and measuring the mandible. The most consistent error found in the metric data was measuring the mandibular angle, where the least experienced observer had 11 instances of being exactly 10 degrees different, indicating measurement reading errors.

The morphoscopic and metric variables are reliable and valid, though recognition of PREI is the most problematic. The morphoscopic traits had moderate agreement between observers, which is related to observer experience. The most variable metric trait between observers was TML (10.53% TEM), which may relate to problems when teeth obstruct a superiorly derived measurement. Overall, the metric measurements among observers have high agreement. In using the methodology presented by Berg, it is suggested that practitioners become sufficiently familiar with the trait definitions and scoring attributes as well as the range of variability in mandibular morphology.

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Anthropology, Reliability, Error of Measurement

A30 Decision Trees and Non-Metric Traits: A More Accurate Approach for Sex Estimation of the Skull

Natalie R. Langley, PhD, Lincoln Memorial University, DeBusk College Osteopathic Med, 6965 Cumberland Gap Parkway, Harrogate, TN 37752; Alesia Cloutier, MS, Lincoln Memorial University, DeBusk College Osteopathic Med, 6965 Cumberland Gap Parkway, Harrogate, TN 37752; Cade Lampley, MS, Lincoln Memorial University, DeBusk College Osteopathic Med, 6965 Cumberland Gap Parkway, Harrogate, TN 37752; and Beatrix Dudzik, PhD, 250 S Stadium Hall, Knoxville, TN 37996*

After attending this presentation, attendees will gain an appreciation for the use of different combinations of non-metric traits of the skull and the utility of predictive decision trees. Combined, these approaches provide a more accurate and simpler method than has traditionally been used by anthropologists.

This presentation will impact the forensic science community by providing a modified scoring system and data manipulation approach that performs variable selection to achieve maximum accuracy with sex estimation. Decision trees are an attractive statistical approach as they can handle numeric and categorical data and do not require normal distributions. As the non-metric scoring systems so often implemented by anthropologists often encompass these parameters, this approach can be incredibly useful.

The use of non-metric categorical scoring methods to estimate sex in skeletal remains is frequently implemented by forensic anthropologists. Traits of the skull have long been a staple in building the biological profile and associated methods are almost always cited in official reports. The most commonly used method of determining sex from the skull is an ordinal scoring system developed by Walker.[1] Despite its widespread use by practitioners, validation studies have indicated that all traits are not equally useful in sex estimation.[2-4]

Low accuracy rates have been attributed to high variability, inadequate categories, and/or definitions for specific traits. Despite obvious flaws with the Walker traits, few proposals have offered a solution to improve the system. Using the decision tree approach allows for quantification of the predictive power of included variables and provides insight into which traditional non-metric traits may not be of value.

Six cranial traits were scored on 220 American skulls of European descent (n=110 males and 110 females) from the UT William M. Bass Donated Skeletal Collection. The traditional variables of the nuchal crest, mastoid process, supra-orbital margin, glabella, and mental eminence were implemented. Additionally, the zygomatic extension was also included, which is often absent in previous studies. Ordinal scores for each variable were partitioned into training and validation samples and decision trees were created using the Rattle graphical user interface available in R.[5,6]

Preliminary results indicate that high correct classification percentages can be reached with smaller subsets of variables. Glabella was one of the most important discriminatory variables, while the mental eminence showed little significance. This supports the results of recent, related publications. Correct classification percentages were reported at 89%-90% for the validation sample, even when using only one or two traits. Further analyses with larger sample sizes and the possible inclusion of newly defined traits will indicate ultimately what combination of variables can simultaneously maximize prediction and reduce user error. As it has been shown by many studies (including the current work) that the mental eminence shows high levels of variability and low predictive power for sex estimation, it is argued that the trait and scoring method should be redefined in such a way that accounts for the range of morphologies often seen between and among the sexes. Additionally, further expansion on the results reported in this study will include more samples that are representative of other ancestries and thus have more relevance for forensic contexts.

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Sex Estimation, Non-Metric, Decision Trees

A31 Sex Assessment — The Utility of Endocranial Landmark Data

Sean Y. Carlson-Greer, BA, School of Medicine - University of Missouri, One Hospital Drive, Columbia, MO 65212; and Stephen D. Ousley, PhD, Dept of Anthropology/Archaeology, Mercyhurst University, 501 E 38th Street, Erie, PA 16546*

After attending this presentation, attendees will understand the potential applications of 3D digitized landmark data to sex assessment of endocranial skeletal remains, specifically, the creation, definition, and utilization of standardized endocranial landmarks and Interlandmark Distances (ILDs) that can be used to assess sex in cases of fragmentary cranial remains.

This presentation will impact the forensic science community by explaining how this analysis will increase the potential number of cranial measurements that can be taken from a set of remains, and allow for more confident assessments of sex in cases of incomplete or fragmentary materials.

Biological profile is a crucial part of any forensic anthropological investigation, of which sex is one influencing factor. Fragmentation and erosion of skeletal remains acts to complicate both metric and non-metric assessment of skeletal remains and lessen the number of potential measurements that can be taken; however, the cranium offers a unique skeletal structure in that fragmentation allows for direct access to complex surfaces and structures that increases the possible number of direct measurements.

Use and definition of ectocranial landmarks have a long history dating back more than a century. In general, these landmarks are now well defined, and their use in everyday craniometrics is standard practice. On the other hand, endocranial structures are rarely defined in terms of landmarks. When endocranial landmarks are identified, their definitions typically lack specificity and leave their exact location open to interpretation. This lack of specificity increases the potential of measurement error and incorrect assessment when data from different samples or studies are collated.

Over the past decade, researchers, including Isaza et al. and Kalmey and Rathbun, have begun to investigate metric sex differences of endocranial structures.[1,2] These studies have shown promise for the use of these structures for sex assessment, both using traditional caliper measurements and computer assisted ILDs; however, previous research has focused on isolated areas within the cranium, leaving out overall morphology or uses landmarks that are methodically difficult to locate and identify.

The present study examined 330 crania from the Rainer Osteological Collection in Bucharest, Romania. Nine midline and 11 bilateral landmarks were defined and collected from the crania using a MicroScribe® G2X digitizer. Landmarks were excluded on an individual basis in cases of fragmentation, erosion, or expression of diffuse or localized pathological conditions. ILDs were extracted for all possible landmark combinations, and subsets of regionally clustered landmarks were created to simulate potential areas of cranial fragmentation. Each set of ILDs was analyzed using discriminant function analysis.

Of the 465 possible interlandmark distances across the entirety of the endocranial surface, a subset of 11, selected in a stepwise analysis, rendered cross-validated classification accuracies up to 85% with an almost negligible observed sex-bias below 5%. Simulated fragmentary analysis of four regionally clustered sets of landmarks results in cross-validated classification accuracies of up to 75% with a sex-bias below 5%. While the classification accuracies reported here are lower than those reported for traditional ectocranial landmarks, they are still appropriate for reliable and statistically sound assessments, particularly of fragmentary materials. Unlike ectocranial landmarks, endocranial structures that are well suited for landmarks are unaffected by muscular attachments that can greatly increase sexual dimorphism of a region or of measurements. While the endocranium has been mostly ignored in terms of sex assessment, with the exception of the petrous portion, it is a complex 3D surface that has great potential in expanding the number and types of analyses that can be used for assessment of the biological profile.

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Biological Profile, Sex Assessment, Endocranium

A32 Femoral Neck Axis Length (FNAL): Use in Sex and Ancestry Estimation of Hispanic Populations

Audrey Murchland, BS, 25 Perth Court, Springboro, OH 45066; Lori E. Baker, PhD, Baylor University, Forensic Research Lab, One Bear Place, #97173, Waco, TX 76798-7173; and Rebecca Meeusen, MS, 42531 Rockrose Square, Unit 302, Ashburn, VA 20148*

After attending this presentation, attendees will be familiar with the FNAL measurement as well as how FNAL can now be used to estimate sex from skeletal remains in Hispanic populations.

This presentation will impact the forensic science community by expanding the use of the FNAL method in sex determination for Hispanic populations. This method will facilitate the identification process for deceased immigrants of Hispanic ancestry on the United States southern border.

Since 1998, the bodies of more than 6,300 deceased undocumented immigrants have been discovered along the United States-Mexico border.1 The majority of these individuals are of Hispanic ancestry. Traditional sexing methods, using standards developed from skeletal collections of American Whites and American Blacks, do not accurately sex those of Hispanic ancestry. In fact, 53% of Hispanic males are misclassified as females using these standards.[2]

Recent studies examining FNAL defined the measurement as the distance from the base of the greater trochanter (the point directly inferior to the greatest lateral projection of the greater trochanter) to the apex of the femoral head, excluding any lipping on the fovea capitus femoris.[3,4] Meeusen measured the FNAL from skeletonized remains of American Black, American White, and Native American samples of both sexes and showed that the FNAL measurement classified ancestry with low accuracy rates, ranging from 41.6% to 48.5%, and classified sex with high accuracy rates, ranging from 84.5% to 87.0%.4

In this study, the FNAL measurement was examined to determine its potential use for sex and ancestry estimation of Hispanic populations. FNAL was measured on skeletally mature adults, void of visible anomalies potentially affecting the measurement. Data was collected from Undocumented Border Crosser (UBC) cases recovered from Falfurrias, TX, (*n*=58: F=21, M=37) by the Reuniting Families Project. These data were then compared with the data from Meeusen: 87 (F=32, M=55) American Black; 108 (F=54, M=54) American White; and 91 (F=44, M=47) Native American individuals.

Statistical analyses were conducted in SPSS and included one-way Analysis of Variance (ANOVA), stepwise Discriminant Function Analyses (DFA), cross-validated sectioning point classification rates, and Bayesian analyses. In addition, a random subset of 55 femora was measured in a second trial for the purpose of intra-observer error assessment. Results showed low intra-observer error, with a Technical Error of Measurement (TEM) of 0.32mm and a coefficient of Reliability (R) of 0.99. A random subset of nine femora was also measured in a second trial for the purpose of inter-observer error assessment. Results showed low inter-observer error, with a TEM of 0.53mm and a R of 0.99, confirming previous repeatability assessments of the measurement.

One-way ANOVA revealed significant differences in FNAL between ancestral groups, with American Whites having the largest FNAL, followed by American Blacks, UBCs, and then Native Americans. Post-hoc Tukey HSD comparisons showed that American White FNALs are not significantly different from American Black FNALs (P=0.881), and UBC FNALs are not significantly different from Native American FNALs (P=0.998). DFAs classified samples by ancestry poorly, with accuracy rates ranging from 34.7% to 40.4%.

One-way ANOVA results also showed significant differences in FNAL between sexes, with male FNALs being significantly larger than female FNALs (P <0.001). DFAs classified samples by sex well, with accuracy rates ranging from 83.4% (ancestry-pooled) to 91.4% (UBC only). The UBC sectioning point, calculated at 87.5mm, provides a classification accuracy of 90.5% for females and 89.2% males.

The FNAL measurement has not been previously applied to Hispanic populations for either sex or ancestry estimation. Using FNAL measured from skeletonized femora, the 87.5mm UBC sectioning point correctly predicts sex at a rate of ~90%. Due to the skeletal variability seen in UBCs and their unidentified status, this sample provides only preliminary information about Hispanic populations. Further collection and analysis of Hispanic skeletal remains is encouraged.

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Femoral Neck Axis Length, Sex Determination, Hispanic

A33 A Multiple Classifier System Approach to Determining Ancestry of Fragmentary Remains: A Preliminary Study

Amber M. Plemons, BS, Mississippi State University, 206 Cobb Institute of Archaeology, Mississippi State, MS 39762; Nicholas P. Herrmann, PhD, Mississippi State University, Cobb Inst Archaeology, Box AR, Dept of Anthro & Mid East Cultures, Mississippi State, MS 39762; and Edward F. Harris, PhD, University of Tennessee, Memphis, Dept of Orthodontics, College of Dentistry, 875 Union Avenue, Memphis, TN 38163*

After attending this presentation, attendees will understand how to utilize a multiple classifier system to best estimate ancestry from human skeletal remains and to select the best-suited methods of ancestry estimation when analyzing fragmentary remains.

This presentation will impact the forensic science community by encouraging a holistic approach to determining ancestry by combining extensive theoretical and methodological research and data collection of previous researchers in attempts to increase reliability of ancestry estimations in the future.

The goal of this research was to assess the most commonly available indicators of ancestry in a fragmented skeletal collection and test a statistical framework for combining all available methods.

Various methods for determining ancestry have been explored, most notably cranial and postcranial metrics, cranial morphology, dental metrics, and dental nonmetric traits, yet little effort has been made to combine these methods for comprehensive ancestry estimation.[1] Furthermore, the current approaches are heavily dependent on complete or mostly complete skeletal remains. The most frequently used methods of ancestry estimation are cranial and postcranial metrics, which are classified via Discriminant Function Analysis (DFA) in the FORDISC® software. Such customized software packages allow for the input of partial datasets; however, these methods are rarely feasible when dealing with fragmentary remains.

This study focuses on combining dental metrics, dental non-metric traits, and cranial macromorphoscopic traits to assess the ancestry of 67 individuals from the Mississippi State Asylum (MSA) Cemetery in Jackson, MS. The asylum records divided the institutionalized population into two classifications of social race (White and Black) and, therefore, a binary classification was developed using European and African American reference datasets. Cranial and postcranial metrics were not used for this study due to the high degree of fragmentation and poor preservation. Individuals with no dentition represented were excluded as this only leaves cranial macromorphoscopic traits to be assessed, reducing the sample to 57 individuals. Dental metrics for each MSA individual were entered into FORDISC® 3.0 and referenced to a dataset of American Blacks and Whites from the University of Tennessee College of Dentistry, Memphis, TN, using the custom import feature in order to calculate posterior and typicality probabilities.[2] Posterior probabilities were then calculated for each individual using dental non-metric traits referenced to frequencies developed by Edgar.[3] Finally, cranial macromorphoscopic were assessed using seven traits established by Hefner.[4] All observed macromorphoscopic traits were incorporated into a Bayesian classifier to calculate posterior probabilities, but this method was limited due to fragmentation. The group of posterior probabilities from the dental metrics, dental non-metric traits, and macromorphoscopics was then averaged, where each classifier was weighted equally.

When comparing the ancestry classifications across methods, 38 of the 57 individuals (66.67%) had consistent classifications. Of the 38 individuals with consistent ancestries, 84.21% had average posterior probabilities greater than 0.75. While there does not appear to be any significant correlations between the consistency of classification across methods and the number traits or metrics used, there is a strong positive correlation between the number of dental non-metric traits used and the posterior probability (r=0.43, p=0.0008). The limited sample size for macromorphoscopic traits rendered correlation tests insignificant; however, it should be noted that the combination of characteristics likely determines accuracy of the methods rather than quantity of metrics and non-metric traits. A discussion will be provided on the availability of each characteristic used to classify the fragmentary remains and trends in combinations of variables, as well as their correlations, within each classifier for all cases of consistent classifications. Additionally, the utilization and issues encountered when dealing with cranial macromorphoscopic traits will be presented.

Jantz and Hefner recommend that researchers embrace the theory of forensic race estimation by providing empirical data engrained with concepts of human variation.[5] This study is an example of such research targeted at creating appropriate and reliable statistical methods for determining ancestry of unidentified remains, as well as an aid in improving the establishment of biological profiles of fragmentary remains.

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Ancestry, Fragmentary Remains, Multiple Classifier Systems

A34 Widening the Scope and Expanding the Field: An Argument for Sociocultural Anthropology's Seat at the Table

Sarah Wagner, George Washington University, Dept of Anthropology, 2110 G Street, NW, Washington, DC 20052*

After attending this presentation, attendees will understand how sociocultural anthropological studies of forensic science, particularly forensic anthropology applied in contexts of ongoing violence and post-conflict communities, elucidates the co-constituting relationship between science and society.

This presentation will impact the forensic science community by fostering dialogue among members of its subdisciplines, especially physical and sociocultural anthropology, about the general field and its theoretical momentum concerning issues pertinent to the recovery and postmortem identification of missing/unknown persons.

Taking forensic anthropological investigations into human rights abuses and missing persons populations as the point of departure, this presentation argues for a more nuanced, theoretically informed understanding of how forensic science is executed and how its results affect lives and communities.[1] In doing so, this presentation builds on two conversations already begun within the Anthropology Section of the American Academy of Forensic Sciences (AAFS): the 2014 decision to change the section name from "Physical Anthropology" to "Anthropology," in which members acknowledged that sociocultural anthropologists were actively contributing to the general field through ethnographic analyses of forensic work; and the set of presentations on "Theory in Forensic Anthropology" delivered at the 2015 AAFS Annual Scientific Meeting. These initial discussions underscored the importance of considering the scope and application of forensic anthropology and which theoretical questions underpin its work.

This presentation goes further, asserting the basic premise that science is never apolitical; rather, it is situated within a specific sociohistorical context and bound up in co-productive relations of political will, social values, cultural practice, and economic conditions.[2] Two examples support this claim. The first is drawn from the forensic efforts to identify the 30,000 missing persons from Bosnia and Herzegovina, specifically the more than 8,000 Bosnian Muslim (Bosniak) men and boys missing as a result of the Srebrenica genocide, where scientific success has yet to translate into enduring sociopolitical repair, despite the goals of its international sponsors.[3] In the Srebrenica case, tensions between individual (and individuated) identity and collective ethnonational identity are often exacerbated during the annual mass burial of identified victims in the communal cemetery of the Srebrenica-Potočari Memorial. Data culled from 12 years of ethnographic analysis of the identification efforts and its results, including the 20th anniversary of the genocide, July 11, 2015, document this tension.

The second example is of the United States military's decades-long efforts to account for its service members listed as Missing In Action (MIA) and presumed dead from the major conflicts of the past century. Recent attempts at reorganizing the government agencies tasked with MIA accounting make manifest the politics of national commemoration influencing not only the inner workings of forensic science but also how these results are perceived by the wider public.[4] Debates surrounding externally dictated quotas for annual identifications, acceptable margins of error, and the role of forensic anthropology in the scientific process expose the politics of a cost-efficient model of MIA accounting.

In examining the co-productive relations between science and society (postwar Bosnia and Herzegovina and contemporary United States), this presentation demonstrates that forensic anthropological efforts to document human rights abuses and/or recover and identify missing or unknown persons cannot be separated — analytically or practically — from the sociopolitical and economic conditions in which they unfold. In failing to recognize the contingent nature of knowledge production, scientists, policy makers, and the wider public risk overlooking, in particular, the political consequences of these forensic efforts.

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Sociocultural Anthropology, Missing Persons, Politics

A35 The Social Process of a Forensic Identification

Hugh H. Tuller, MA, Defense POW/MIA Accounting Agency, 310 Worchester Avenue, Joint Base Pearl Harbor-Hickam, HI 96853-5530*

After attending this presentation, attendees will have a fuller understanding and appreciation of how the social interactions between scientific and non-scientific actors can shape the forensic identification process.

This presentation will impact the forensic science community by exploring the nature of objective scientific practice in human identification through the lens of Science and Technology Studies, which theorizes that much of scientific work is far from neutral, value-free observation. Understanding this nature will better prepare the field of forensic anthropology to mitigate effects of bias and lead to more comprehensive identifications.

This presentation seeks to expand the conversation on theory in forensic anthropology introduced during last year's AAFS Annual Scientific Meeting. In particular, this presentation will engage with Winburn's analysis of knowledge production within the field, further interrogating the view that positivist science is value-free and separate from the social context in which it is produced.[1] Rather, this presentation argues that the scientific process of identification itself is a cultural artifact. As such, its results represent a truth accepted as a "black box" — the inner workings of which are known to be complex, but that complexity is not necessary to understand.[2] In reality, the process is not only scientifically sophisticated, but also profoundly affected by social interaction and subjectivity where discrepancies and potential bias are ignored or downplayed.

As an example of Winburn's critique, this presentation will demonstrate how the forensic identifications produced at the Defense POW/MIA Accounting Agency's Central Identification Laboratory (DPAA-CIL) are based on amalgamations of decisions and interpretations scientists make in the course of their examinations and tests, the interactions they have with their scientific peers, supervisors, and non-scientists, as well as the influences of historical, political, and economic factors. These interactions are partly the nature of the work that requires interactions with outside laboratory actors and partly built into the protocols and standard operating procedures of the institution. Together, they form a particular DPAA-CIL cultural viewpoint and an approach to the work that often goes unexamined and unchallenged.

The process of a forensic identification and indeed the production of scientific knowledge are usually perceived as a neutral truth-finding practice that discovers facts through the unbiased testing of hypotheses or application of proven methods and techniques. This process is viewed and even trumpeted as one devoid of social influence, where the production center of knowledge is like a citadel whose walls prevent biased influences from the rest of the world.[3] In this manner, science is seen as cultureless by both the lay public and scientists themselves.[4] Embracing this view, most forensic anthropologists involved in the identification of an unknown individual subscribe to the belief that a positive identification is the result of sterile, neutral, stand-alone science; however, as the example of DPAA-CIL reveals, an identification involves the review by the CIL's Scientific Director of a compilation of specialized reports authored by both scientists and non-scientists. Moreover, each report is an end product of a series of scientific tests or observations created within a context of various social interactions and potentially mitigating political and economic factors.

The field of Science and Technology Studies has demonstrated that ideologies of scientific neutrality and objectivity are poor guides to how science is actually made.[5] This example of the DPAA-CIL's forensic identification process attempts to challenge the cultural assumptions about the scientific process and to push the attendee to peek into the black box and examine not only the complexity of the science, but the contingencies under which those tests and observations are made. Shining a light on these conditions demonstrates how a forensic identification is as much a social as a scientific process. Most importantly, greater awareness of how identifications are made will allow forensic practitioners to better mitigate biases and improve their identification processes.

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Forensic Identification, Anthropological Theory, Scientific Process

A36 The Social Side of Human Identification

Robin C. Reineke, PhD, University of Arizona, 1009 E South Campus Drive, Tucson, AZ 85721*

After attending this presentation, attendees will understand how context-specific sociocultural anthropological expertise can support and enhance the applied work of human identification, especially in violent or post-conflict settings. Focused as it is on social worlds and local contexts, sociocultural anthropology can provide a bridging or mediating role between forensic practitioners and local communities.

This presentation will impact the forensic science community by providing evidence and examples of effective collaboration between forensic practitioners and sociocultural anthropologists in the realm of human identification.

Observations and data used in this presentation come from more than nine years of applied forensic work and research among forensic practitioners at the Pima County Office of the Medical Examiner (PCOME) and families of missing and deceased migrants along the United States-Mexico border. Data are drawn from the Colibrí Center for Human Rights, as well as from interviews with forensic scientists, law enforcement, border patrol agents, non-profit family advocates, and the families of missing and deceased migrants.

This presentation is focused on a specific set of findings, namely that forensic identification processes that fail to take into account political, cultural, and social contexts are not only less effective but may actually cause harm. As victims of violence are often structurally vulnerable, care must be taken so that forensic investigations are undertaken with an awareness of the political context and translated with cultural sensitivity.[1] Without efforts to integrate the family and community into the process, forensic identifications can become medicalized, reproducing violent structures enforced by those in power and disenfranchising the healing process that drives so many forensic practitioners to do the work they do. Medicalization is the process whereby normal human conditions become problems for medical professionals in a manner that often has long-term negative social health consequences.[2] In this presentation, the concept of medicalization is applied to examine forensic practices of human identification: can forensic investigations, especially in violent contexts, serve to medicalize grief in a way that exacerbates the trauma and suffering of affected communities?

Results of long-term participant observation reveal that forensic identifications can indeed cause additional suffering and trauma for families, especially if the identification and notification process is conducted with an approach that is not sensitive to community context and history. Some of the best models for avoiding these problems involve collaboration between sociocultural anthropologists and forensic anthropologists in international human rights (post-conflict) settings. Research for this presentation reveals that there are needs, as well as precedent, for such collaborative approaches within the United States domestic context.

The PCOME is one of the first offices in the nation to integrate local knowledge in a meaningful and sustained manner. By working with social scientists and community advocates, forensic practitioners at the PCOME have been able to revise and improve protocols so they align more closely with family and community needs.[3] The PCOME works closely with the Colibrí Center for Human Rights, a family advocacy organization founded by cultural anthropologists. Colibrí manages an antemortem database relevant to missing migrants and communicates directly with families throughout intake, investigation, and case resolution. Colibrí attends to the social side of identification, which includes explaining all aspects of the investigation to families, fielding their questions, and offering supportive advocacy grounded in a place of understanding and respect for the family's needs. The results of this collaboration include more than 100 successful identifications. Partnerships such as that between the PCOME and Colibrí model a best practice where efforts are made to link the "affective identification" made by families with the scientific identification made by forensic practitioners.[4]

The scientific process of human identification cannot be entirely separated from the social context in which it operates. If the family does not trust, understand, or have a stake in the work of forensic practitioners, positive identifications will do nothing to assist families and communities in healing. There is an entire other side to the identification process that is critical — the social side of identification. If the social side is not connected to the scientific side of identification, forensic work can become imposed on communities in harmful ways. Collaboration between forensic practitioners and sociocultural anthropologists is offered as a step forward in both international and domestic contexts.

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Identification, Migration, Sociocultural Anthropology

A37 Family Opposition to Human Rights Exhumations: The Need for Interdisciplinary Research on a Question of Science, Politics, and Consent

Adam R. Rosenblatt, PhD, Haverford College, 370 Lancaster Avenue, Haverford, PA 19041*

After attending this presentation, attendees will understand how ethnographic, historical, and political science research can clarify the reasons why some family members have opposed the exhumation of mass graves and identification of missing persons, even in cases in which those efforts are framed as "transitional justice" work and fueled to a great extent by concern for the needs of these very families.

This presentation will impact the forensic science community by addressing a topic that some experts allege "has been largely, shamefully avoided in forensic anthropology literature": the objections of a key group of stakeholders, families of the missing, to the exhumation and identification of their dead.[1] It is a crucial topic because of the ways in which it pits three priorities widely acknowledged as important — the creation of an objective historical record, the collection of evidence, and the needs of families of the missing — against one another.[2] On the occasion of the 2014 decision to change the name of the Physical Anthropology Section to "Anthropology" and the 2015 opening of the AAFS Humanitarian and Human Rights Resource Center, this presentation illustrates a crucial area in which new interdisciplinary connections can be made between the practical challenges of forensic science in the human rights context and emerging sociocultural studies of the needs of families of the missing in post-conflict regions.

Based on eight years of research, including archival research in two languages and interviews with forensic anthropologists, human rights activists, and religious leaders, this presentation identifies two broad categories of objections to post-conflict exhumations: religious and political; however, in this process, it argues for a nuanced understanding of the connections between the two: religious objections can serve political purposes and political objections can be infused with notions of the sacred.

While cultural and religious objections to both exhumation and autopsy can impact death investigation in any context, these objections are particularly complex in post-conflict areas in which forensic science comes to be seen not only as mechanism for medicolegal truth, but also as a "technology of repair" and of memory for divided societies.[3,4] In Poland and the Democratic Republic of the Congo, forensic anthropologists have faced not only religious prohibitions against exhumation, but also complex questions about the extent to which the religious leaders and community members who were making these objections spoke for the interests of all families of the missing or for all survivors of violence. As they made decisions about whether and how to proceed with exhumation efforts, they were hampered by a lack of supporting sociocultural research into the political pressures that informed these religious objections and other questions of power and representation in these communities.[5]

Other types of objections, more political in thrust, are unique to the circumstances of human rights and transitional justice — particularly because of the ways in which human rights violations organize networks of victims and systematize their claims.[6] The first and still best-known mobilization against forensic investigations into human rights violations occurred in Argentina, starting in the mid-1980s, when Clyde Snow and the Argentine Forensic Anthropology Team began exhuming the graves of "disappeared" victims of right-wing political repression. The anthropologists faced protests from Argentina's most famous group of human rights activists, the Madres de Plaza de Mayo, who demanded that legal accountability for the perpetrators of violations be prioritized before any form of identification, mourning, or "closure."[7] The Madres' organizing slogan, "Aparición con vida" ("Let the disappeared alive") lent the appearance of irrationality to a group of activists whose family members were, except for a few extraordinary cases, almost certainly dead.[8]

This presentation joins other scholars in finding it unacceptable to dismiss anti-exhumation sentiment — religious or political — as the irrational demands of "superstitious" cultures or of people traumatized by grief.[9,10] It calls for both data-sharing across context and sociocultural analysis of the widespread phenomenon of these objections in order to promote informed critical engagement. The reasons for family members' views on exhumation are multi-dimensional — political, moral, historical, and sometimes scientific — and often contain both important insights and misrepresentations. Only through both comparative and deeply contextual understandings can forensic anthropologists be adequately prepared for principled negotiation of this important challenge.

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Human Rights, Families, Anti-Exhumation

A38 Morphological and Metric Study of the Nose and Ear in a North Indian Population: Forensic Anthropological Context

Kewal Krishan, PhD, Panjab University, Dept of Anthropology, Sector 14, Chandigarh 160 014, INDIA; Tanuj Kanchan, MD, Dept of Forensic Medicine, Light House Hill Road, Mangalore, Karnataka 575 001, INDIA; and Manojit Chakraborty, MSc, Panjab University, Department of Anthropology, Chandigarh 160014, INDIA*

After attending this presentation, attendees will understand the importance of the variability in appearance of the nose and ear in a north Indian population, which will also strengthen knowledge in facial forensic identification of victims of mass disasters and in crime scene investigations.

This presentation will impact the forensic science community by presenting new information on the uniqueness and variability in the appearance of the nose and ear in a north Indian population and their usefulness in forensic facial identifications.

Facial reconstruction is an important aspect of forensic anthropology which helps in establishing the identity of the deceased or the perpetrator of a crime. Facial reconstruction can be achieved by a forensic scientist/facial anthropologist using the original skull, a replica, or clinical images of the skull. Another method of facial reconstruction involves photographic records and Closed-Circuit Television (CCTV) images of the various features of the face wherein a forensic artist reconstructs the face with the aid of additional information gathered from acquaintances. Ear and nose patterns provide important and useful information for facial reconstruction. When dismembered and mutilated human remains are recovered, the individualistic features of the nose and ear can help in the identification of the deceased.

The present study was conducted with a view to evaluating metric, morphological, and unique features of the nose and ear among young adults in a north Indian population. The data were collected from a sample of 215 participants (104 males and 111 females) between 18 years and 25 years of age. The study evaluated the inter-individual variation and sex differences in morphological and metric features of the nose and ear. The morphological features of the nose and ear, such as nasal root, nasal bridge, nasal profile, nasal septum, nostril shape, nasal wings, shape and size of the ear, shape, size, attachment, and thickness of the ear lobe, shape of the tragus, helix, Darwin's tubercle, and hypertrichosis, were examined in this sample. General metric measurements of the nose and ear were gathered for baseline data.

The results indicate that the overall dimensions of the nose and ear in males were found to be significantly larger than females. Bilateral variations were observed for some of the measurements. No significant sex differences were found in the nasal index (males=64.98, females=65.57). Similarly, the left and right ear indices were not significant (for the left ear, males=57.53, females=56.40; for the right ear, males=56.49, females=55.49). The morphological parameters of the nose were found to be quite variable in both sexes. Significant variations existed in nasal profile, nasal tip, nasal septum, nostril shape, and thickness of nasal wings. For the morphological parameters of the ear, oval-shaped ears were quite common (80.77% in males and 72.07% in females). A squarish-ear lobe (41.34% in males and 74.77% in females) was more frequently observed than other types. Attached ear lobes were found in 55.77% of males and 82.89% of females. A normally rolled helix was present in 62.50% of males and 79.28% of females. Darwin's tubercle was found among 5% of the study population. Hypertrichosis was observed in approximately 13% of the males.

Facial Identification, Nose and Ear, North Indians

A39 Morphologic Analysis of the Location of the Lens on the Orbit Using 3D Reconstructed Models

Dong-Ho Eddie Kim, BSc, 222 Banpo-daero, Seocho-gu, Seoul 137-701, SOUTH KOREA; Yi-Suk Kim, MD, PhD, Ewha Womans University, Dept of Anatomy, School of Medicine, 911-1, Mok5-dong, Yangcheon-gu, Seoul 158710, SOUTH KOREA; Dae-Kyoon Park, MD, PhD, Soonchunhyang University, Department of Anatomy, College of Medicine, 31 Sooncheonhyang 6-gil, Dongnam-gu, Cheonan-si, Seoul 31151, SOUTH KOREA; In-Beom Kim, PhD, The Catholic University of Korea, 222 Banpodaero Seochogoo, Seoul 137701, SOUTH KOREA; and U-Young Lee, MD, The Catholic Univ of Korea, Dept of Anatomy, Coll of Med, 505, Banpo-dong, Seocho-gu, Seoul 137701, SOUTH KOREA*

After attending this presentation, attendees will understand the sex differences found using 3D models and landmark coordinates for eyeball placement and the development of regression formulas for lens protrusion. In addition, morphometric characteristics of the orbit according to sex and the use of regression formulas to find the location of the lens center for use in forensic facial reconstruction will be presented.

This presentation will impact the forensic science community by illustrating the sex differences in orbital morphology and a reliable location of the lens for fontal and lateral views. In addition, the regression formulas are developed in this study to find the most probable lens protrusion location.

The goal of this research is to study the relationship between the lens location and the orbit-related structures for eyeball placement in forensic facial reconstruction. A total of 200 high-resolution cranial Computed Tomography (CT) scans were studied. The sample was composed of 100 men and 100 women, with age ranges of 21years to 70 years; the overall mean age was 46 years. The 3D cranium and lens models were reconstructed from the Digital Imaging and Communications in Medicine (DICOM) data using the Mimics® version 16.0. Ten distinct landmarks were indicated on the cranium and lens models. A total of 25 morphological and angular measurements between landmarks including the lens center were measured by the Mimics® software and were analyzed by the Statistical Package for the Social Sciences (SPSS) version 20.0.

The study results describe general orbit morphology and interpret the relationship between orbit-related structures, including the lens center. First, there were sex differences in the orbital morphology and these results match other data of generalized orbital-morphologic differences in males and females. Males had more developed eyebrows and a more receding inferior orbital rim. In addition, males had a bigger orbit and the orbit was rotated in a more clockwise manner. Second, there were no sex differences in lens location; the location of the lens can be estimated regardless of sex. On the frontal view, the lens can be placed at the point of 55.59% of Medial Orbitale (MO)-Lateral Orbitale (LO) breadth horizontally and 48.40% of Superior Orbitale (SO)–Inferior Orbitale (IO) height perpendicularly. On the lateral view, the lens can be placed approximately 1.11mm in front of Orbitale Tangent Plane (OTP) line. In addition, more sophisticated methods using regression formulas can be used to estimate the lens protrusion. The presented first formula is the traditionally used regression formula that is modified for use in Korean populations. The next two formulas are proposed in this study to improve the reliability of lens protrusion, as the first formula has a low $R^{2\ value}$. The morphometric characteristics of the orbit, including the lens center and the regression formula to estimate the location of the lens center, will be helpful for forensic facial reconstruction.

Lens Location, Orbit, Facial Reconstruction

A40 A Challenging Case of Facial Reconstruction of a Suicide by Jumping From a Height

Luigi Cipolloni, MD, PhD, Viale Regina Elena, 336, Rome 00161, ITALY; Alessandro di Luca, MD, Via Domenico Chelini 7, Rome 00197, ITALY; and Laura Donato, Via Tripolitania 195, Rome 00199, ITALY*

After attending this presentation, attendees will better understand an experimental, alternative method of facial reconstruction that substitutes artificial supports for missing or degraded bony elements in cases of heavily disfigured victims.

This presentation will impact the forensic science community by showing how this method of facial reconstruction could be useful in cases where the bony elements of the face are completely destroyed and are unsuitable for facial identification.

In a forensic context, the process of identification is complementary to the forensic examination. In cases of highly decomposed or skeletonized unknown decedents, the intervention of the forensic anthropologist can provide additional information about age at death, sex, and other parameters that cannot be easily detected due to the lack of soft tissues.

A crucial part of the identification process is comprised of the facial reconstruction: this procedure is based on rebuilding the soft tissues and the physiognomic general aspect of the victim's face. The skull represents the skeletal support, and standard soft tissue depths are applied to specifics landmarks. The result is a 2D or 3D representation of the features belonging to the non-identified body. The skeletal tissue supplies a solid support whereby tissue depth can be placed: by these means, it is possible to consider the variability of the skull morphology and the rendering of the application of standard tissue depth.

The present study was challenged by the case of a 33-year-old woman who committed suicide by jumping from a window of her flat. Her face was heavily disfigured by the impact from the fall. The main trauma was directly to the head and this completely destroyed the facial features. The bony supports of the cranium were fractured and impossible to reconstruct, as various fragments were also missing. Only the mandible was present, but was broken into two pieces; however, it did allow for some observations of the morphological structures of the chin.

In order to reconstruct the anatomic distribution of the facial tissues, an artificial support was used to simulate the missing bone structures. In classical reconstruction techniques, the process performed is usually just the opposite — artificial soft tissues are fixed onto the bone structures. In this case, the landmarks had to be fixed on the soft tissues and then applied onto a solid support, a polystyrene form simulating a human head. The edges of lacerated skin were stitched and fixed on the structure with pins. After the facial reconstruction, the result was photographed: graphical elaboration was necessary in order to delete the evidence of lacerated skin and make it suitable for identification purposes.

The creation of an artificial support, substituting the skull, allowed for the rebuilding of the physiognomic facial traits, yielding a positive comparison result to a photograph of the possible subject. Furthermore, the facial reconstruction supplied additional information about the exact site where the subject's head impacted the ground.

Forensic Anthropology, Facial Reconstruction, Identification

A41 The Perceived Accuracy of 3D Facial Reconstructions

Eileen M. Schilling, MSc, 613 Cayman Avenue, Holly Springs, NC 27540*

After attending this presentation, attendees will be aware of the implications of possible identification from 3D facial reconstructions.

This presentation will impact the forensic science community by showing that leads generated by 3D facial reconstructions should be viewed with caution and are generally unreliable.

Often 3D facial reconstructions are a final effort to lead to the identification of skeletal or decomposed remains of an individual, when all other methods have failed to provide leads to identification. Generally, 3D facial reconstructions are considered to be inaccurate because their accuracy relies on the connection between the reconstruction and a living individual. This study tested the accuracy of 3D facial reconstructions in three ways: (1) by assessing the perceived accuracy of a facial reconstruction when compared to a known individual; (2) assessing the consistency of reconstructions created using the same protocol; and, (3) assessing the consistency of the raters.

For this study, 11 different participants each created one facial reconstruction from a cast of the same known individual. Each of the participants completed the reconstructions following the same protocol. After the reconstructions were completed, photographs were taken of each reconstruction. The experiment was evaluated in two parts: (1) a likeness rating of each reconstruction against an array of seven individuals of similar age, race, and sex as the known individual; and, (2) biometric comparison of the reconstructions to the known individual. For the likeness ratings, each rater was asked to rate each of the reconstructions against the array of photographs and provide a score from one (the reconstruction looks nothing like the person) to ten (the reconstruction is of that person). After these ratings were completed, the raters were informed of which photograph was the known individual and asked to rate the reconstructions again, but against only the known individual. Five standard biometrics measurements were taken: forehead to tip of the nose, tip of the nose to the chin, distance between middle of the eyes, width of the mouth, and total length of the face.

The ratings data was analyzed using a Kendall's coefficient of concordance. This allowed a determination of inter-rater agreement for each of the reconstructions. All reconstructions except one showed statistical significance ($p<0.05$), indicating that there is agreement between the scores assigned to each reconstruction from the different raters. For one reconstruction, there was no agreement among the raters. Inter-rater reliability for the photograph of the known individual, while the individual remained unknown to the raters, was tested. None of the reconstructions returned statistically significant results against the known, with significance levels of $p\geq0.993$. The ratings acquired after the known individual was revealed to the raters showed similar inconsistencies in the ratings, with significance levels of $p\geq0.990$. Subsequently, the biometric measurements were analyzed using a one-sample *t*-test. Only one of the measurements, the width of the mouth, was not statistically different ($p=0.406$) from the measurement of the known individual. The other four measurements showed statistical significance ($p<0.05$), indicating that those measurements are statistically different from the known individual's measurements.

The results address two main issues with facial reconstructions. The first is that without an accurate initial outline of the face, any evaluation of the accuracy of the reconstruction cannot be considered to be a true indication of its accuracy. The second issue is how individuals perceive a face cannot truly be tested if the initial face (i.e., the reconstruction) is too inaccurate to be recognized as the individual it is intended to be. Since facial reconstructions rely on individuals recognizing a face they are familiar with, the fact that none of the raters knew the individual they were supposed to be identifying may have hindered their recognition of the reconstructions as that person. Further research should focus on the accuracy of the initial outline of the face to increase accuracy of overall facial reconstructions.

Facial Reconstruction, Accuracy, Perceived Accuracy

A42 Application of Enhanced Point Estimators on a Sample of *In Vivo* Computed Tomography (CT) -Derived Facial Soft Tissue Thicknesses

Kelsey Kyllonen, MA, 2501 Investigation Parkway, Quantico, VA 22135; Connie L. Parks, MA, Federal Bureau of Investigation, 2501 Investigation Parkway, Laboratory Division, Quantico, VA 22191; and Keith L. Monson, PhD, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, attendees will better understand the level of congruence between the conventional arithmetic mean and the shorth and 75-shormax statistics for facial soft tissue depths.

This presentation will impact the forensic science community by providing the results of further investigation into the conventional use of average facial soft tissue depths and the applicability of an alternative statistical technique for measuring central tendency. Additionally, this presentation will provide support for the standardization of best practices in regard to facial soft tissue research.

Facial approximation refers to the process of approximating an antemortem representation of an unidentified individual from his or her skull. This representation is commonly achieved by applying and modeling clay directly to the individual's skull or a skull replica while utilizing the bone morphology as a construction guide. Facial soft tissue depth measurements are a key component in the development of an effective facial approximation. Soft tissue depth research has an extensive and productive history, encompassing more than a century of investigation and employing an expansive array of collection methodologies, analyses, and populations. One common strategy across facial soft tissue depth research is the use of the arithmetic mean as the primary central tendency descriptor. While the arithmetic mean is an informative statistic, some researchers argue that it is not a robust descriptor of central tendency in datasets exhibiting skewed distributions and thus may not reveal the true attributes of a tissue depth dataset. The purpose of this study is to illustrate application of the facial soft tissue depth analysis tool TDStats R to a contemporary American tissue depth dataset and explore the level of congruence between the arithmetic mean, shorth, and 75-shormax statistics.[1,2]

Facial soft tissue depth measurements were collected from cranial Computed Tomography (CT) scans of 388 living American adults.[2] The scans included males (n=198) and females (n=190) from four self-identified ancestry groups ranging in age from 18 years to 62 years (mean: 31, median: 30). Although ages and weights varied considerably, no individuals were eliminated from the study. Two experienced researchers independently collected 14 midsagittal and 11 bilateral soft tissue depths for each CT scan. Summary statistics, including the shorth and 75-shormax, were calculated for the 25 tissue depths using TDStats R.[1] Differences between arithmetic mean and shorth values for the 25 tissue depths ranged from 0.1mm to 2.3mm (average 0.6mm), with no difference exceeding one Standard Deviation (SD) of the mean. In addition, differences between the arithmetic mean and 75-shormax values for the tissue depths were approximately one SD of the arithmetic mean for many tissue depth points, and none exceeded two SD. Differences between the arithmetic mean and 75-shormax mean tended to be greatest for the midfacial region.

These findings suggest that the mean and shorth values of the study sample are congruent within ±1 SD. The results also indicate that 75-shormax values include ±1 to 2 SD of the mean. Although no practical difference between the arithmetic mean, shorth, and 75-shormax statistics was observed in this tissue depth dataset, the statistics may still prove beneficial for analysis of tissue depth datasets. Although shorth values may better represent skewed distributions, they will regress to the arithmetic mean when applied to normally distributed data.

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Facial Approximation, Facial Reconstruction, Tissue Depths

A43 Evaluation of the Facial Soft Tissue Thickness in the Living in a Brazilian Population: Pilot Study

Antonio A. Antunes, PhD, Rua Cardeal Arcoverde, 267, Graças, Recife, Pernambuco, BRAZIL; Hugo L. Albuquerque, Faculty of Dentistry - University of Pernambuco, Av. General Newton Cavalcanti, s/n, Camaragibe, BRAZIL; Evelyne P. Soriano, PhD, Faculty of Dentistry, University of Pernambuco, Av. General Newton Cavalcanti, s/n, Camaragibe, BRAZIL; Marcus Vitor D. Carvalho, PhD, Faculty of Dentistry, University of Pernambuco, Av. General Newton Cavalcanti, s/n, Camaragibe, BRAZIL; Reginaldo I.C. Campello, PhD, Faculty of Dentistry, University of Pernambuco, Av. General Newton Cavalcanti, s/n, Camaragibe, BRAZIL; and Gabriela G. Porto, PhD, Faculty of Dentistry, University of Pernambuco, Av. General Newton Cavalcanti, s/n, Camaragibe, BRAZIL*

After attending this presentation, attendees will better understand creating a database reference thickness of the soft tissues in the living population of Recife, Brazil, according to age, sex, and Body Mass Index (BMI).

This presentation will impact the forensic science community by providing results on the possibility of forming a database that can help forensic anthropological practices and procedures of human identification, more specifically for the identification of human skeletal remains. As a result of this method, it would be possible to acquire an image of the unknown individual based on average values of the soft tissue thickness in specific populations obtained, which can allow the recognition of an individual.

Currently, there is a huge demand for human identification, as well as decedent or skeleton recognition. Several methods of identification are described in each of these situations, depending on if the subject is alive or dead or is in a cadaverous or skeletal stage.[1] Thus, for human skeletal identification remains, a variety of methods are cited, such as DNA analysis and tooth radiographs.[2] Although these methods provide important information for forensic scientists about age, sex, and deceased body size, many may not be useful because they depend on the availability of a comparative material, either from police, dentists, or relative's databases.[3] Facial reconstruction is the last option when other identification methods have failed. Data on the soft tissue thickness represent an integral part of the paths to obtain the approximation of the individual's face.[4] Previous studies show that different populations exhibit significant variation in the thickness of the soft tissue being questioned and whether data of a population may be applied in facial reconstruction with different ancestry.[5,6] Thus, for obtaining an accurate facial reconstruction, the construction of a database of soft tissue thicknesses to a specific population is required. In the Brazilian population, literature is scarce and there are only records of studies dealing with cadavers.[6]

A cross-sectional and prospective study was performed. Data collection was conducted in patients from the Oswaldo Cruz Hospital, University of Pernambuco (HUOC/UPE) radiological clinic who had Computed Tomography (CT) scan examinations. Collected CTs were measured for facial soft tissue thicknesses in 20 selected craniometric points: supra-glabella, glabella, nasion, rhinion, midphiltrum, supra-dental, infra-dental, supra-mental, pogonion, mentual, supra-orbital, infra-orbital, lateral-orbit, inferior zygomatic, zygomatic arch, supra-glenoid, gonion, supra-M2, occlusal line, and sub-M2. For the comparison between categories of independent variables in relation to means, Student's t-tests with equal variance were used, Student's *t*-tests with unequal variances or Mann-Whitney tests in cases of comparisons between two categories were undertaken, and the *F*-test Analysis of Variance (ANOVA) or Kruskal-Wallis comparisons were used for more than two categories.

The total sample consisted of 30 patients, 15 men and 15 women, aged between 12 years and 78 years old. The mean age was 42.93 years, with slightly more than half (53.3%) 40 years of age or older. Most individuals had brown skin color, followed by 23.3% of the sample who were Black and 10.0% who were White. The average weight, height, and BMI were 69.57kg, 1.65m, and 25.53, respectively. The two highest percentages corresponded to those who were overweight (36.7%) and eutrophic (33.3%) and the lowest corresponded to those who were malnourished (10.0%). It was observed that with the exception of the distances or measures D9, D13, D15, D18, and D20 that had higher averages in females than in males, the other average measurements were correspondingly higher in males, though with significant differences between the sexes ($p<0.05$) in distances D1, D3, D4, D5, D6, D7, D13, and D17. Regarding BMI, the study showed that the measure D9 (pogonion) was the only one with significant differences; the average was lower among the malnourished (7.81), followed by the eutrophic (9.78), and the range was 11.85 to 12.63 between the obese and overweight, with significant differences between the malnourished and those who were overweight and obese and among the eutrophic with those who were overweight.

It can be concluded that significant differences between age groups for any of the evaluated measures and between the sexes ($p<0.05$) in distances D1, D3, D4, D5, D6, D7, D13, and D17 were present. Only one point (D9) varied significantly from BMI.

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Soft Tissue Thickness, Computed Tomography, Facial Reconstruction

A44 Applications of 3D Technology in Forensic Anthropology

Helen Cho, PhD, Davidson College, PO Box 6934, Davidson, NC 28035; Eun Jin Woo, PhD*, Seoul National University, Dept of Anthropology, San 56-1, Silim-dong, Kwanak-gu, Seoul, SOUTH KOREA; Hae Joung Cho*, MAKRI in Hyunchungwon, Dongjack-gu, Seoul, SOUTH KOREA; Yu Ryang Jang, PhD*, 65 Hyeonchung-ro Donggak-dong, Donggak-gu, Seoul 156-080, SOUTH KOREA; and Nahyok Im, PhD*, 65 Hyeon Chung-Ro, Dong Jak - Gu, Seoul 156-080, SOUTH KOREA*

After attending this presentation, attendees will better understand the different types of 3D technology and their applications in forensic anthropology and related forensic disciplines.

This presentation will impact the forensic science community by providing results that compare various 3D scanning systems and the quality of the models produced. This presentation encompasses a relatively new type of technology and novel applications in forensic anthropology and related disciplines such as forensic art.

With the advent of 3D technology, the potential applications are numerous in the forensic sciences. Forensic anthropology and related disciplines, such as forensic art, can benefit from 3D imaging technology and 3D printing of skeletal remains. One of the main objectives of forensic anthropology is to reconstruct the biological profile of the unknown skeletonized individual. Whether the remains are positively identified and should be returned to the next of kin, or unidentified and kept in custody, the bony remains may be needed for anthropometric analyses and facial reconstruction. Both 2D photography and scanned images may be insufficient for further anthropometric analyses if the original remains are unavailable for pragmatic reasons. Performing facial reconstruction on the actual skull may be impermissible, and utilizing the real human remains as physical models in court while serving as an expert witness may be deemed unethical and disturbing to the audience. Furthermore, rare anatomical and anthropological specimens can be reproduced for educational purposes. Thus, 3D technology can be a necessary tool for forensic anthropologists to create replicas of the skeletal remains.

In this regard, the most important issue is the quality of the 3D model. Anthropometry requires precise measurements of standardized osteological landmarks, and these quantified data are then employed to derive the biological profile of the unknown individual. The quality of 3D replicas is dependent on image resolution and digitizing systems. Although digitizing natural bone at a high resolution will result in a more precise model, access and availability of the best digitizing system and the 3D printer may be limited to organizations such as law enforcement and universities. In this study, the quality of the anatomical models created from a single 3D printer using various digitizing systems was compared.

Three different techniques were employed to digitize a skull at the Central Identification Laboratory in the Ministry of National Defense in Seoul, Korea. Three separate systems were printed with the Zprinter® 650 powder-based 3D printing technique utilizing a powder composed of plaster and a starch/cellulose mixture at the speed of 28mm per hour. The digitizing systems were as follows: (1) Computed Tomography (CT); (2) an ATOS 1 (0.8M) 3D scanner with software that captures the image in an 800,000-pixel triangle mesh; and, (3) a PHT-6500 panorama X-ray with a rotating X-ray tube that captures a panoramic image of the object. To test the quality of the replicas, 34 standard cranial measurements (e.g., nasal height, bigonial width, mandibular angle) were collected from the original skull and the CT, ATOS 1, and PHT-6500 models for comparison.

Based on the results of this study, although CT scans produce the highest resolution images in general, it may be unnecessary to rely only on CTs when comparable technology is available and more accessible.

3D Printing, 3D Scanning, Anthropometry

A45 Automated Anthropometric Measurements of Long Bones Using Point Cloud Data

Lisa M.M. Van Den Broek, Weg Naar Geneuth 121, Maasmechelen 3630, BELGIUM; Thera McAvoy, MSc, PO Box 8945, Albuquerque, NM 87198; Roland Wessling, MSc, Cranfield Univeristy, Cranfield Forensic Institute, Shrivenham, Oxfordshire SN6 8LA, UNITED KINGDOM; Jessica Bolton, MSc, Cranfield University, Cranfield Forensic Institute, Defence Academy of the United Kingdom, Shrivenham SN6 8LA, UNITED KINGDOM; Jelana Bekvalac, MSc, Museum of London, 150 London Wall, London EC2Y 5HN, UNITED KINGDOM; Anja Leipner, Institute of Forensic Medicine, University of Zurich, Winterthurerstrasse 190/92, Zurich 8057, SWITZERLAND; and Michael Thali, MD, Universitat Zurich, IRM / Forensic Institute, Winterthurstrasse 190/52, Zurich CH-8057, SWITZERLAND*

After attending this presentation, attendees will understand the current and future potential for traditional osteometry to be enriched by the application of software capable of producing automated osteometric measurements, thus reducing the risk of intra- and inter-observer error.

This presentation will impact the forensic science community by demonstrating that 3D point clouds created from both dry bones and medical Computed Tomography (CT) scans can be modeled and automatically measured. This proof of principle strongly indicates that future development in this field will allow the majority of traditional anthropometric measurements to be made with software programs either fully or semi-automatic, without the need of manual intervention. These measurements will be repeatable, reproducible, and can overcome the lack of universal measurement standards within forensic anthropology, which makes it specifically relevant for the forensic aspect of anthropology.

Osteometry is widely used and accepted in the forensic, archaeological, and anthropological world. This is to establish a biological profile, which typically includes sex, age, stature, and ethnicity of an individual by using regression formulas that require measurements of various bones and bone regions. The accuracy of these measurements is partially determined by how much experience the practitioners have, as well as where each practitioner chooses to take measurements, as there are no clearly defined or universally accepted best practices.[1-3]

With current technology, it is possible to create detailed 3D models of bones and perform measurements on them. Previous studies show that measuring 3D models, produced with CT scanners or hand-held laser scanners, are equally as accurate as when physically measuring on a dry or fresh bone.[4,5]

Two studies were performed, one with 31 dry femora from the St. Brides Church Crypt collection in London, England, and one using 40 femora from CT scans provided by the Institute of Forensic Medicine at the University of Zurich, Switzerland. The maximum length was obtained from the dry bones with an osteometric board and manually from 3D models of the CT scans in MeshLab. The maximum length of the point clouds was then obtained automatically within custom software. Due to the pioneering nature of these studies, multiple methodologies for data handling and analysis were developed and tested in order to determine their feasibility prior to the creation of the custom software.

The results of these studies show clear correlations, which provide evidence that measurements taken automatically by software are as good as those taken manually, and thus has the potential to be used for biological profiles. This would have the advantage of allowing practicing forensic anthropologists to present easily reproduced and quantifiable results.

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3D Models, Automated Measurements, Virtual Skeletal Analysis

A46 Introducing Standardized Anthropological Measurement Protocols for Postcranial Bones Using 3D Surface Reconstructions in Computed Assisted Design (CAD) Software

Mikaela S. Reynolds, MSc, Level 5 Q Block,, 2 George Street, Gardens Point, Brisbane, Queensland 4001, AUSTRALIA; Donna M. MacGregor, MSc, Queensland University of Technology, School of Biomedical Sciences, Faculty of Health, Gardens Point Campus, Brisbane, Queensland 4001, AUSTRALIA; Mark D. Barry, MS, Queensland University of Technology, High Performance Computing and Research Services, 2 George Street, Brisbane, Queensland 4001, AUSTRALIA; Nicolene Lottering, BS, Queensland University of Technology, School of Biomed Sci, Faculty of Health, 2 George Street, Gardens Point, Brisbane, Queensland 4001, AUSTRALIA; and Laura S. Gregory, PhD, Queensland University of Technology, School of Biomedical Sciences, Gardens Point Campus, Brisbane, Queensland 4001, AUSTRALIA*

After attending this presentation, attendees will appreciate the improved accuracy and reliability attributed to semi-automated anthropological measurement protocols on 3D reconstructions of postcranial bones using CAD software.

This presentation will impact the forensic science community by demonstrating the benefits of Multi-Slice Computed Tomography (MSCT) and the advances of a virtual approach as a non-invasive method for obtaining reproducible morphometric information in anthropological investigation. The visualization and measurement capabilities of reverse engineering software will be discussed. The protocol introduced in this study and the precision testing results presented are essential for advancing current medicolegal death investigations, accentuating the advantages of "virtual anthropology."

Consistent with the International Criminal Police Organization (INTERPOL) Disaster Victim Identification Guide, postmortem identification during mass disasters systematically involves the utilization of MSCT. Specifically in Australia, postmortem MSCT was regarded as a valuable tool in disaster victim identification during the 2009 Victorian Bushfires and constitutes standard protocol for external autopsy in a number of Australian mortuaries. The 3D surface-rendered models in CAD software has the potential to increase measurement accuracy in comparison to Multi-Planar Reformatted (MPR) assessment, which uses contiguous 2D orthoslices, where the outer boundary of macroscopic bony landmarks may be arduous to determine. Utility of MPR models also requires a considerable level of anatomical imaging knowledge, as the investigator is required to mentally construct a 3D representation from 2D images.

The goal of this present study was to introduce a contemporary osteometric protocol using CAD software and to conduct observer error testing to assess the reliability of this protocol. Six thin-slice Digital Imaging and Communications in Medicine (DICOM) datasets (thickness: 2mm, overlap: 1.6mm, voxel size: 0.78mm x 0.78mm x 2.0mm) of the femoral region of contemporary adult Australian individuals (aged neonate to 75 years old) were accessed from the Skeletal Biology and Forensic Anthropology Virtual Osteological Database. The femora were subject to manual segmentation to produce an isosurface model compatible with the engineering software program Geomagic Design X™ for osteometric examination. In Geomagic Design X™, the principal component axes were realigned for the construction of a series of anatomical reference planes required to depict a "virtual osteometric board." With reference to silhouette curves, extreme position planes corresponding to the outermost boundary of the isosurface are identified in order to obtain automated plane-to-plane measurements. Bicondylar length and epicondylar breadth were measured by four observers differing in CAD software experience over three separate days to evaluate intra- and inter-observer error. Technical Error of Measurement (TEM), relative Technical Error of Measurement (%TEM), and Intraclass Correlation Coefficient (ICC) were calculated to quantify the measurement error variance and observer agreement of the protocol.

Intra- and inter-observer error results demonstrate that the linear measurement protocol introduced is highly repeatable. Specifically, intra-observer error resulted in %TEM=0.10, ICC=1.000 (CI=0.999-1.000) for bicondylar length, %TEM=0.19, ICC=0.995 (CI=0.980-0.999) for epicondylar breadth. Inter-observer error resulted in %TEM=0.50, ICC=0.995 (CI=0.978-0.999) for bicondylar length, %TEM=0.20, and ICC=0.996 (CI=0.979-1.000) for epicondylar breadth. Since these results are within the acceptable levels of agreements for anthropometric measures, it is recommended that this protocol be implemented in anthropological casework and contemporary anthropological research.

The protocol introduced in this study utilizes high-quality 3D models, which allow "hidden features" such as the medullary cavities to be observed, with the software also providing the opportunity for novel geometric morphometric methods to be developed. A further benefit of CAD software is the use of automated plane-to-plane measurements, which eliminates the requirement of manual identification of landmarks, also reducing the subjectivity associated with the alignment of the bone in MPR protocols.

As it is crucial that all contemporary scientific methods are validated and standardized, this study introduces a technologically new, standardized protocol for linear measurement of postcranial bones using 3D surface reconstructions, with observer error testing demonstrating strong observer agreement. It is therefore suggested that 3D isosurface models using CAD software can be utilized for osteometric assessment of human remains. In addition, this presentation will emphasize the wide application of this protocol, demonstrating standard anthropological measurement of other skeletal elements of the postcranial skeleton (e.g., humerus and scapula) inclusive of subadult long bones.

MSCT, Reverse Engineering, Observer-Agreement

A47 Virtual Skeletal Analysis (ViSA) — One Possible Future for Osteometrics

Roland Wessling, MSc, Cranfield Univeristy, Cranfield Forensic Institute, Shrivenham, Oxfordshire SN6 8LA, UNITED KINGDOM; Sophie Beckett, PhD, Cranfield Forensic Institute, Defence Academy of the United Kingdom, Shrivenham, Swindon, Wiltshire SN6 8LA, UNITED KINGDOM; Jessica Bolton, MSc, Cranfield University, Cranfield Forensic Institute, Defence Academy of the United Kingdom, Shrivenham SN6 8LA, UNITED KINGDOM; Alice Jenny Butcher, BSc, Cranfield University, Cranfield Forensic Institute, Shrivenham, Oxfordshire SN6 8LA, UNITED KINGDOM; Lisa M.M. Van Den Broek, Weg Naar Geneuth 121, Maasmechelen 3630, BELGIUM; and Thera McAvoy, MSc, PO Box 8945, Albuquerque, NM 87198*

After attending this presentation, attendees will better understand what has already been achieved in moving from physical osteometrics and qualitative anthropological assessments to virtual, digital, and quantitative analysis. Furthermore, the current and future opportunities and possibilities in this field will be outlined to enable attendees to develop their own research in this area.

This presentation will impact the forensic science community by detailing the processes involved in ViSA and the considerable range of low and high equipment, software, and methodologies involved. This will show that digital bone analysis can be performed with very few and affordable facilities and that the real challenge is the development of entirely new methodologies and in not acquiring complex hardware and software.

Osteometry and its use in establishing biological profiles has long been researched and practiced by anthropologists. Traditionally, it is done on physical, dry bones or bone regions using calipers and osteometric boards. Landmarks on bones are identified by the anthropologist and used to measure and record the various distances that can be used to determine, or at least estimate, age, sex, stature, and ancestry.

Through the development of imaging technology, from digital cameras to Computed Tomography (CT) scanners, a new way of doing osteometrics has been developed. There are two main processes involved: scanning and data analysis. The scanning or data creation can be accomplished in various ways, such as photogrammetry, laser or light scanning, and CT scanning. Different methods have different advantages and disadvantages.

The raw data then needs to be processed to create a 3D representation of an actual bone or bone region. Various software packages are being used for this and much is currently being done manually; however, research is being carried out to establish reliable automated processes to make this aspect far more efficient. The virtual bone model can then be analyzed and this is currently being done with either software that was written for a different purpose, such as Geographic Information Systems software or software that is written specifically for research projects. Some researchers carry out their osteometric measurements manually on the virtual bone while others attempt to develop software that detects bones, bone regions, and landmarks automatically or semi-automatically and then perform the osteometric measurement equally automatically.

While the idea of not using an actual, physical bone and not measuring it with an equally real tool will no doubt irritate some anthropologists and evoke considerable opposition, there are a number of advantages to be considered, some of which are: (1) scanned bone data files can be stored permanently with far less logistical effort compared with real skeletal remains; (2) virtual bone models can be produced from the living or from decedents who are fully fleshed; (3) population-specific data could be produced for any region on Earth from living populations; (4) analysis of virtual bones can easily be shared with other scientists, without the need to travel long distances; (5) sample sizes for research projects could be increased considerably by sharing resources between institutions; (6) some virtual data can show the inside of bones, not only the outside; (7) automatic osteometric measurements would be more accurate and far more repeatable with no inter- or intra-observer error; and, (8) automated systems could produce far more measurements that can be taken virtually rather than physically.

The full potential of ViSA will only be revealed when anthropologists start thinking outside the box and outside of the current, restricted ways in which osteometrics are performed. There is far more information in the bones that can only be unlocked digitally.

Virtual Skeletal Analysis, Osteometrics, 3D Bones

A48 Incorporating the "Black Bone" Magnetic Resonance Imaging (MRI) Technique: A Radiation-Free Alternative to Computed Tomography (CT) for Biological Profiling in the Living

Janamarie Truesdell, MSc, University of Oxford, School of Anthropology and Museum Ethnography, 51/53 Banbury Road, Oxford, Oxfordshire OX2 6PE, UNITED KINGDOM; Karen A. Eley, DPhil, University of Cambridge, Dept of Radiology, Addenbrooke's Hospital, Hills Road, Cambridge, Cambridgeshire CB2 0QQ, UNITED KINGDOM; Anthony McIntyre, BS, Oxford University Hospitals, The Churchill Hospital, Old Road, Headington, Oxford OX3 7LE, UNITED KINGDOM; and Nicholas Márquez-Grant, PhD, Cranfield University, Cranfield Forensic Institute, Defence Academy of the United Kingdom, Shrivenham SN6 8LA, UNITED KINGDOM*

After attending this presentation, attendees will be familiar with a novel, non-ionizing Magnetic Resonance (MR) technique with the potential to eliminate the need for CT in future biological profiling research.

This presentation will impact the forensic science community by introducing the radiation-free "Black Bone" MRI sequence, which, when 3D rendered, provides diagnostic image quality equivalent (for the purposes of age estimation) to that of CT scanning.

For forensic anthropologists, called upon for investigations involving both the living and the recently dead, the future does not lie in the past but in the present. To make the most appropriate profiling estimations, a constantly updated understanding of modern populations and how they change is of paramount importance; however, as reference collections, and he techniques and assumptions based upon them, become increasingly removed from the people of today, this becomes more and more difficult with each passing year. In order to remain knowledgeable about the populations that constitute the bulk of the anthropologist's casework, it is imperative that we begin to update our reference material as quickly and as comprehensively as possible. To do this, an intensified focus into medical imaging is required. Bone is optimally imaged by CT; however, due to ethical considerations regarding ionizing radiation exposure, imaging studies have been limited to patients already being examined for other purposes. This precludes the recruiting of volunteers for specific investigations such as the effect of substance abuse on aging from the fourth rib. For such a study, individual substances would have to be separated so as not to confuse results (smokers often drink and drinkers often smoke, so two groups — a smoking-only group and a drinking-only group — would have to be recruited to isolate these variables). This type of tailoring is currently impossible due to the restrictions surrounding CT, effectively stifling advancement in this area until an alternative can be found.

"Black Bone" MRI offers a potentially revolutionary solution, achieved through a novel approach, to overcome the notorious failures of routine MRI techniques in 3D bone imaging. Utilizing a gradient echo sequence with a low flip angle (optimized to 5°), short Repetition Times (TR=8.6ms) and Gradient Echo times (GE=4.2ms), "Black Bone" MRI enhances the soft tissue-bone interface by reducing the contrast of the surrounding soft tissues and medullary bone. This results in bone appearing densely black (hence "Black Bone") while the surrounding tissue remains a uniform gray, thus lending itself to 3D bone-rendering techniques.[1] "Black Bone" MRI has been utilized in a range of clinical settings, particularly within the head and neck, with successful 3D bone reconstruction. Recognizing the forensic potential of this technique, a small pilot study was focused upon reconstructing the pubic symphysis to investigate its applications for biological profiling.

Five patients undergoing multimodality (both MRI and CT) imaging of the pelvis for routine clinical care at Oxford's Churchill Hospital, United Kingdom, were recruited. "Black Bone" MRI images of the pubic symphysis were 3D rendered utilizing a range of image-processing techniques. Age estimation was performed on both the "Black Bone" MRI and CT images using the Suchey-Brooks method for aging the os pubis.[2] Results for both modalities were then compared and correlated with known age. In each case, "Black Bone" MRI proved an equally suitable medium for age estimation from the pubic symphysis.

In conclusion, this pilot study demonstrates the promising, immediately applicable advantages of the "Black Bone" MRI technique both in current forensic context and as a much-needed potential replacement for CT in future biological profiling research.

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Biological Profiling, Medical Imaging, Living Participants

A49 DCP 2.0: Changes in Data Collection Procedures for Forensic Skeletal Material

Natalie R. Langley, PhD, Lincoln Memorial University, DeBusk College Osteopathic Med, 6965 Cumberland Gap Parkway, Harrogate, TN 37752; Lee Meadows Jantz, PhD, University of Tennessee, Dept of Anthropology, 250 S Stadium Hall, Knoxville, TN 37996-0720; Shauna McNulty, MA, University of Tennessee, 250 S Stadium Hall, Knoxville, TN 37996; Heli Maijanen, PhD, University of Oulu, PO Box 1000, Oulu 90014, FINLAND; Stephen D. Ousley, PhD, Dept of Anthropology/Archaeology, Mercyhurst University, 501 E 38th Street, Erie, PA 16546; and Richard Jantz, PhD, University of Tennessee, Dept of Anthropology, Knoxville, TN 37996-0720*

After attending this presentation, attendees will be aware of changes to skeletal measurements that interface with the FORDISC® software program. Attendees will be provided information about Data Collection Procedures for Forensic Skeletal Material 2.0 (DCP 2.0) and an accompanying online instructional video. Additionally, attendees will be surveyed about the need for continuing education in osteometric data collection.

This presentation will impact the forensic science community by informing practitioners and researchers about significant changes in osteometric data collection protocols related to the FORDISC® software and ongoing data collection efforts of the Forensic Data Bank. This information is imperative for forensic anthropologists who use the FORDISC® software in their forensic casework.

The foundations for changes in osteometric data were presented at the 2015 AAFS Annual Scientific Meeting (Abstract A84) and the preliminary results of research that is now complete. Briefly, the National Institute of Justice funded an effort to determine error rates associated with commonly used skeletal measurements and to evaluate alternatives for problematic measurements. Four observers took 78 standard (34 cranial and 44 postcranial) and 20 less-commonly used measurements on 50 skeletons from the University of Tennessee's William M. Bass Donated Collection. Each observer repeated the measurements on the 50 skeletons four times with a two-month time lapse between sessions. Relative Technical Error of Measurement (TEM), Scaled Error Index (SEI), and repeated measures Analysis of Variance (ANOVAs) with Tukey-Kramer post-hoc tests were used to assess the degree of inter-observer and intra-observer error for each measurement. Final results indicate that measures of maxima and minima are the most precise and repeatable measurements between and among observers. For example, biauricular breadth and bizygomatic breadth have relative TEM values of 0.054 and 0.0719, respectively. On the other hand, ischium and pubis length have respective TEM values of 36.52 and 41.06, respectively. SEI values showed that ischium and pubis length also have high intra-observer error rates. Repeated measures ANOVAs were used to determine measurements that were overall poor performers versus measurements that performed well with two or more observers (but not across the board). Inconsistent error rates between observers were indicative of problems with measurement definitions.

The final results of this research have brought about the following changes in osteometric data: (1) positionally dependent measurements of shaft diameters (i.e., sagittal, transverse, dorso-volar, anterior-posterior, etc.) have been abandoned for maxima and minima at midshaft for all long bones; (2) pubis and ischium length have been removed; (3) several measurements of epiphyses and/or articular surfaces have been added (e.g., maximum olecranon breadth, maximum radial head diameter, Anterior-Posterior (AP) diameter of S1, maximum glenoid cavity breadth); and, (4) landmark and measurement definitions have been clarified to facilitate higher precision and repeatability. Most definitions follow Howells and Martin and Knussmann; the latter was checked for translation accuracy.[1,2] Error rates for all measurements will be provided in the DCP 2.0 manual. The manual will be available in portable document format online and as a bound laboratory or field manual. In addition, an accompanying instructional online video will be made available to practitioners. Work is in progress to make the video available in English, Spanish, and French.

The DCP 2.0 is designed to be compatible with the FORDISC® software, as this software will become the primary means of collecting skeletal and osteometric data for research and development purposes. As such, the age estimation material in the DCP is being replaced with the transition analysis method. Materials have been provided to be incorporated into the DCP 2.0. Also, the new DCP will be versioned, like the associated FORDISC® software, to ensure that users are apprised of updates.

The information reported in this abstract does not represent the opinions of the National Institute of Justice.

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Osteometric Data, Observer Error, FORDISC®

A50 Scanning Electron Microscopy/Energy Dispersive X-Ray (SEM/EDX): A Rapid Diagnostic Tool to Aid the Identification of Burnt Bone and Contested Cremains

Sarah Ellingham, MSc, Teesside University, School of Science and Engineering, Middlesbrough TS1 3BA, UNITED KINGDOM*

After attending this presentation, attendees will have a new appreciation for the value of SEM/EDX analysis for forensic anthropological investigations, particularly for the analysis of burnt remains.

This presentation will impact the forensic science community by detailing how the SEM/EDX can act as a quick and minimally destructive "fingerprinting" tool depicting a sample's elemental composition, thus aiding in the identification of burnt bone and contested cremains.

Having been recognized as a valuable tool to forensic anthropologists since the 1980s, the SEM has found application in a variety of different anthropological scenarios. SEM lends itself to the analysis of osseous material for numerous reasons; it has a superior resolution of 3D structures and a greater depth of field than light microscopy as well as being able to achieve higher magnification. When combined with EDX analysis, an insight into the gross elemental distribution of the sample can be directly related to a visual image of the assessed specimen. This makes it a useful screening tool when the osseous origin of a sample is in question, as was the case in the 2002 Tri State crematory incident. Although this technique has sporadically found application to burnt bone investigations, there has to date been no published, systematic study evaluating the change of EDX spectra over different exposure temperatures or whether the presence or absence of soft tissue during incineration plays a role.

Fresh sheep (O*vis aries*) ribs were divided into two experimental groups, defleshed and fleshed, with fleshed samples retaining the abdominal wall, circa 2cm subcutaneous fat and skin. Triplicates of each sample group were burned in an electric muffle furnace for 45 minutes at temperatures between 100°C and 1,100°C in 100°C increments. All remaining soft tissue was removed post burning. Samples were subsequently analyzed on a tabletop SEM, using a Backscatter Electron (BSE) detector, fitted with an EDX spectrometer for elemental analysis. Use of the variable pressure setting eliminated the need for sample coating. Morphological information was obtained using the BSE detector at 50x and 100x magnification. EDX measurements were taken at a live time of 50sec with a voltage of 20kV, mapping all detectable elements. For each experimental condition, nine EDX measurements were taken. Some trace elements such as Si and Al were removed from the spectra prior to analysis as they did not appear uniformly throughout samples and represented bones of one individual rather than elemental abundance, which was investigated in this study. A three-factor Analysis of Variance (ANOVA) was performed on the atomic percentage of each element in the sample.

Visual analyses of the external bone surface did not find any discernable differences between bones which were burnt with and without soft tissue present. Contrary to the findings of other researchers, curved "thumbnail" fractures were observed in defleshed as well as fleshed bones at high temperatures. Results of the 3-factor ANOVA showed that neither the exposure temperature nor whether the bones had been burned with or without soft tissue present made any significant influence on the bone's overall elemental makeup ($p>0.05$). The Calcium to Phosphorus (Ca/P) ratio, which several researchers refer to as the most characteristic elemental signature of bone, lay within the literature-quoted values of between 1.6 and 2.58 for both fleshed and defleshed bones when calculated using their weight percentage. There was no trend or pattern in these values at different temperatures.

This study has demonstrated that, even when faced with high temperatures, the overall gross elemental content and atomic percentage of elements in bone remains more or less stable, thus creating a unique "fingerprint" for osseous material, even after having been exposed to extreme conditions. The presence of soft tissue during burning does not change this. SEM/EDX has been found to be a valuable tool in the analysis of burnt bone and lends itself as a fast and minimally destructive method to identify burnt bone from other non-osseous material, which may otherwise appear morphologically similar.

SEM/EDX, Burnt Bone, Forensic Anthropology

A51 Reconstructing the Biological Profile of Cremated Human Remains

Anthony W. Hudson, BS, 9823 Utopia Drive, Apt 5203, Pensacola, FL 32514*

After attending this presentation, attendees will better understand the process of recreating pertinent biological profile information about cremated or burned human remains and which methods prove to be most successful.

This presentation will impact the forensic science community by providing results from a case study in an area with little previous or current research. This presentation will add to research being performed in the fields of forensic taphonomy and forensic anthropology by broadening the understanding of how human remains are affected by a burning episode and how those changes affect the process of identification.

Reconstructing the biological profile of burned/cremated remains can prove to be a difficult task due to the extensive damage that fire inflicts on bone, such as heat-induced fracturing/fragmentation, shrinkage, and shape alterations.[1] The relatively small amount of literature on the topic also serves to make the reconstruction process more difficult. Although there are some methods specific to identifying cremated remains, more standard methods may be required in a situation where remains have incurred damage from fire, whether accidentally or purposefully.

The basis of this research comes from a case study performed in 2013 utilizing an unprocessed commercial cremation, meaning that the remains were not pulverized after cremation, from Western Carolina University's skeletal collection. The remains were screened through a ¼" table screen and a series of nested screens in order to separate the largest and most identifiable pieces for use in reconstructing the biological profile. Approximately 25% of the remains could be readily identified as a certain element with distinguishing features present. These discernable elements provided the basis for the reconstruction process in this study. A multitude of identification methods were utilized throughout this study to assess aspects of biological profile such as sex, stature, and age. Techniques used to assess sex include cremation-specific weight studies performed by Warren and Maples, Bass and Jantz, and Van Deest et al., as well as the more standard Walker method of scoring the greater sciatic notch.[2-4] The Steele method of partial bone length reconstruction was utilized to predict stature, and the Suchey-Brooks and Todd methods utilizing the pubic symphysis to estimate age-at-death were also used.[5] These estimations were then compared to available personal data for the individual in order to assess the success of the various methods applied.

Application of the aforementioned methods proved to be partially successful for this case study, success being defined as having been able to apply a reconstruction technique and have an accurate estimation of biological profile returned. The results were deemed to be partially successful due to the cremation-specific sex estimations yielding various results (male, probably male, and ambiguous), while the greater sciatic notch clearly indicated male. Stature was estimated by applying the Steele method to a fragment of humerus, specifically the head and proximal shaft, yielding a stature between 5'8" and 6'. Living stature for this individual was unknown, so the accuracy of the stature estimation could not be determined. Age estimation based on pubic symphysis morphology produced a broad age range (27 years to 66 years), which was representative of the actual age at death for this individual (57 years).

In conclusion, it was discovered that it is possible to reconstruct, at least partially, the biological profile of human remains that have been burned, even at extreme temperatures. This is important to the fields of forensic anthropology and forensic taphonomy because it demonstrates that there are methods applicable to situations where burning has occurred, whether it is an attempt to conceal identity or a mass disaster.

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Cremation, Taphonomy, Biological Profile

A52 Experimental Analysis of Burned Human Remains

Amanda Williams, MA, 2450 Lymbery Street, Apt 309, Reno, NV 89509; Elayne J. Pope, PhD, Tidewater OCME, 830 Southampton Avenue, Ste 100, Norfolk, VA 23510; and Marin A. Pilloud, PhD, University of Nevada, Reno/0096, 1664 N Virginia, Reno, NV 89557*

After attending this presentation, attendees will better understand the soft tissue and skeletal changes that occur to human remains as a result of a fire. Attendees will learn which variables are strongly correlated and can potentially be used to predict fire conditions.

This presentation will impact the forensic science community by describing the experimental analysis of burned human remains, while also highlighting important variables in fires that alter remains. This work will be the start of creating a new model to investigate fire-related damage to soft and skeletal tissue, which will prove important in aiding investigators who are building a legal case.

Currently, forensic anthropologists use the Crow-Glassman Scale (CGS) to analyze burned remains.[1] This scale progresses quickly from blistering to fragmentation in only five stages, without descriptions of times or temperatures that contribute to these changes. The scale can also be fairly subjective as it does not quantify surface area or percentage of body affected. As such, descriptions of burned bodies from pathologists and medical examiners are often inconsistent with those provided by forensic anthropologists.[2-5] The challenge at hand is to bridge the work between the various practitioners of the forensic sciences, all of whom may examine the same remains. Therefore, this pilot study employs a subset of data that seeks to develop a method more applicable to remains encountered in fires. This initial study employs the CGS as a first step in analysis and identifies variables that are significant in creating the conditions observed. The next step of this study will be to employ a larger dataset with additional variables to develop a more detailed descriptive model of burned human remains.

The initial study involves data collected in 2015. Observational experiments involved the burning of six donated human cadavers as part of the San Luis Obispo Fire Investigation Strike Team training course. Three cadavers were placed in vehicle fires and the other three in structural fires. All physical alterations to both soft and skeletal tissues were documented with digital photography and thermocouples within the fire environment. The visual assessment of the burned bodies was guided by the existing CGS and supplemented by additional descriptions of time and temperatures. Skin splits, subcutaneous fat exposure, muscle exposure, and presence or absence of soft tissue color banding were among the soft tissue variables recorded for each individual. Skeletal color banding, percent fragmented, and percent charring were among the skeletal changes recorded.

Individuals exhibited varying degrees of heat-related damage. The physical alterations were found to differ depending on fire environment, temperature, duration, and location of remains within each context. A majority of the remains from the car fires remained intact, with limited soft tissue loss and bone exposure, consistent with a CGS score of two; however, one individual exhibited calcination on the skull, upper and lower limbs, hands, and feet, which was absent in the other individuals, and is consistent with a CGS score of five. This individual also exhibited heat-related fracturing on both upper and lower limbs, a feature also absent in the other sets of remains. The variability in heat-related damage can be explained by this individual's placement in the trunk, longer exposure to heat, and increased temperatures.

Remains from the structural fires exhibited partial soft tissue loss and soft tissue color banding, which was concentrated at the lower and upper limbs, feet, and hands, consistent with a CGS score of two. None of the individuals in the structure fires exhibited bone exposure or fragmentation as seen with the car fire remains. The structure fire remains also exhibited a larger percentage of soft tissue color banding than the car fire remains. The variability found between vehicular and structural fires is due to variations in the type of environment, followed by the duration and temperatures of exposure.

Overall, the six individuals in this study demonstrate variability in heat-related damage. The initial results demonstrate there are differences between type of fire environment and duration, illustrating the possibility of modeling heat-related conditions. As data collection progresses, a more robust model can be created to predict fire conditions based on damage to soft and skeletal tissues.

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Burned Human Remains, Crow-Glassman Scale, Forensic Anthropology

A53 Patterns of Ossification in Macerated Thyroid Cartilages: Implications for Age and Sex Determination

Katelyn L. Bolhofner, MA, Arizona State University, 900 S Cady Mall, Tempe, AZ 85287; and Laura C. Fulginiti, PhD, Forensic Science Center, 701 W Jefferson, Phoenix, AZ 85007*

After attending this presentation, attendees will be aware that the degree of ossification of the thyroid cartilage should not be used as an indicator in age-at-death assessment.

This presentation will impact the forensic science community by eliminating a commonly used indicator of age at death and by introducing a sex-specific pattern of ossification in the thyroid cartilage.

The ossification process of the thyroid cartilage has been researched extensively using radiographs and Computed Tomography (CT) scans.[1-6] This research has attempted to correlate age at death with the pattern of ossification, but the results are conflicting. Some suggest there is a standard progression that can be sorted into phases useful in age estimation, while others have found little correlation between ossification and age at death. Despite these conflicting results, ossification of the thyroid cartilage continues to be used as an indicator of advanced age. By examining the bone in these cases, it has been demonstrated that there is no correlation with age and no uniform pattern of ossification. Rather, it was found that there does appear to be a recognizable difference in pattern of ossification between the sexes.

This pilot study examined 32 ossified thyroid cartilages removed at autopsy. The sample included 20 males and 12 females that ranged in age from 25 years to 79 years. The samples were collected from modern forensic cases between 2005 and 2015 at the Maricopa County Office of the Medical Examiner and macerated according to standard protocols. Male and female samples were separated and the thyroid cartilages were scored using standard scoring techniques. The published phases were discordant with observed patterns of ossification in this sample, so photographs of the anterior view of the thyroid cartilages were taken and ordered by degree of ossification. Known age at death was then re-associated with the samples.

No statistically significant correlation between degree of ossification and age-at-death was found in either males or females (Spearman's rank-order: male: ρ=0.057, p=0.813, α=0.05 female: ρ=0.224, p=0.484, α=0.05). The highest degree of ossification was observed in a 31-year-old male and one of the least ossified thyroid cartilages was observed in a 79-year-old male. None of the thyroid cartilages from the female samples was as well ossified as those from the male samples. A statistically significant difference in the pattern of ossification was observed between males and females (χ2=8.5, p=0.00349, α=0.05), specifically in the ossification of the superior horns (cornua). Six of 20 males (30%) and 10 of 12 females (80%) exhibit ossification of the superior horns. This element appears to be the last portion of the cartilage to ossify in males, but appears early in the ossification process in females.

The majority of research on thyroid cartilage ossification has been conducted using non-invasive techniques, particularly radiography. Review of this literature demonstrates the difficulty in using these techniques to investigate the cartilage. For example, errors were published in two studies: the first indicates structures in a radiograph that are mislabeled as the thyroid cartilage, the second submits an artistic rendition of the ossified cartilage that is presented upside down.[7-8] Further, in this study, comparison of the digital postmortem radiographs obtained at autopsy to the macerated samples demonstrates that ossification is difficult to appreciate in a radiograph.

The results of this pilot study do not support any correlation between age at death and degree of ossification of the thyroid cartilage. The common perception that the presence of an ossified thyroid cartilage denotes very advanced age is patently false. Practitioners should not rely on ossified thyroid cartilage in any way during an age-at-death assessment. Further, this research demonstrates a significant difference between the pattern of ossification observed in males from that seen in females, suggesting that there may be implications for the use of thyroid cartilage ossification in sex estimation. This difference between the sexes is supported by recent immunohistochemical research that demonstrates sex-specific differences in cartilage mineralization of the laryngeal structures.[9-10] While the results of this pilot study appear to have important implications for skeletal analysis, an increase in sample size should strengthen the findings presented here.

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Thyroid Cartilage, Ossification Patterns, Sex Determination

A54 Age Estimation of Adolescent and Post-Adolescent Children Via Radiographs of the Shoulder

Maureen Schaefer, PhD, Michigan State University, Division of Human Anatomy, E Fee Hall, 965 Fee Road, East Lansing, MI 48824-1316; and Gerald Aben, MD, Michigan State University, Radiology Bldg, 846 Service Road, Rm 184, East Lansing, MI 48824*

After attending this presentation, attendees will better understand the ages at which specific developmental milestones occur in regard to three of the epiphyses surrounding the shoulder. The radiographic data provided within the presentation can then be utilized to estimate the age of living or deceased children. Insight will also be gained into developmental processes that can aid in the determination of whether an individual is likely to be older or younger than two common threshold ages (16 years or 18 years).

This presentation will impact the forensic science community by providing age documentation of a joint region whose development has been traditionally understudied via radiographic means.

Age estimation using radiographic data is becoming increasingly important due to a surge in requests to estimate the age of living children. While numerous radiographic studies have been conducted on the development of the hand, medial clavicle, and iliac crest, a paucity of data exists in the recording of radiographic changes occurring at the shoulder. This presentation fills that void by providing the appearance and/or union times of the epiphyses of the angle/apex of the coracoid process, acromion process, and proximal humerus.

Developmental processes occurring at the three epiphyses were noted utilizing multiple views of shoulder radiographs from 264 males and 189 females between the ages of 10 years and 21 years of age. Images were obtained via two sources, including the Michigan State University's Clinical Center and Query Patricia, an online juvenile radiographic database developed by Mercyhurst University. Each epiphysis was assigned a unique phasing system based on the extent to which developmental processes could be visualized radiographically. Progressive union of the proximal humerus was the least challenging of the elements to interpret and therefore received the most robust phasing system, which included four stages: Phase 1=open union; Phase 2=active union; Phase 3=an unfused notch remains; and, Phase 4=complete union. Appearance times of the proximal humerus were unable to be documented due to the age limitations of the sample, which only included preadolescent and adolescent children. The epiphyses of the coracoid and acromion processes presented a greater interpretive challenge and therefore were assigned phasing systems that were less descriptive; however, the late appearance times of both these epiphyses did permit the inclusion of this event within the phasing system. The acromion process was assigned a three-phase scoring system: Phase 0=epiphysis not present; Phase 1=present and open or fusing; and, Phase 4=complete union. The angle and apex epiphyses of the coracoid process were the most difficult to interpret and therefore information was only recorded if the epiphysis was present and not completely fused.

Observations were recorded for each of the three elements and their age/phase distributions provided. A number of developmental processes were observed to always occur before the age of 16 years or 18 years. These results suggest that the shoulder region may be of particular value when evaluating the likely direction of an individual's age in relation to either of these two common threshold values.

Shoulder Development, Age Estimation, Developmental Osteology

A55 A Grading System to Assess the Sex and Parity Status for the Preauricular Sulcus

Sarah E. Canty, PhD, Liverpool John Moores University, James Parsons Bldg, Byrom Street, Liverpool, Wiltshire L3 3AF, UNITED KINGDOM; Matteo Borrini, PhD*, Liverpool John Moores University, RCEAP-School of Natural Science & Psych, Byrom Street, Liverpool L3 3AF, UNITED KINGDOM; Constantine Eliopoulos, PhD, Liverpool John Moores Univ, School of Nat Science & Psych, James Parsons Bldg, Byrom Street, Liverpool L3 3AF, UNITED KINGDOM; and Silvia Gonzalez, PhD, Liverpool John Moores University, Byrom Street, Liverpool, Merseyside L3 3AF, UNITED KINGDOM*

After attending this presentation, attendees will have a better understanding of the sexual dimorphic variability of the preauricular sulcus in human adult os coxae.

This presentation will impact the forensic science community by providing a grading system for the preauricular sulcus as a sexually dimorphic trait and a musculoskeletal stress marker.

Forensic anthropologists, anatomists, and clinicians have long suggested that pregnancy and parturition may leave a "scar" on the skeleton, especially the os coxae; however, there has been much debate and no clear method for examination has been established. The preauricular sulcus or groove is found on the os coxae between the auricular surface and the greater sciatic notch. It is the site of the attachment for the anterior sacroiliac ligament. During pregnancy, this ligament is loosened in order to widen the birth canal in preparation for parturition; these changes cause remodeling of the bone that can be observed.

The goal of this research was to examine the effects of sexual dimorphism, pregnancy, and parturition on the preauricular sulcus.

Two English medieval skeletal collections were examined in this research, the Poulton collection (sample size=59) and the St. Owens Church Gloucester collection (sample size=108) both housed at Liverpool John Moores University. The skeletal material has been aged and sexed using multiple established methods and compared through inter-observer error with the estimations produced by other forensic anthropologists.

A grading system was designed to examine the different types of sulcus, which was evaluated to range from Grade 0 to Grade 4: (1) Grade 0 — no preauricular sulcus present; (2) Grade 1 — a preauricular sulcus that is shallow and the floor of the sulcus has a uniform depth; there are no pits or grooves and the edges are often undefined. This grade is often scarcely visible, which can make the measurements difficult, as there are usually no definite edges; (3) Grade 2 — a preauricular sulcus whose floor has a slightly uneven depth and is not completely smooth. There should only be a small change in depth; however, a preauricular sulcus with more than one pit of different depths would instead be classified as a Grade 3; (4) Grade 3 — this Grade, although similar to Grade 2, differs as the floor of the sulcu has multiple varying depths and will have more than one pit. Grade 3 has a more defined edge than Grades 1 and 2; and, (5) Grade 4 — a preauricular sulcus whose floor has a very inconsistent depth; it will have multiple deep pits or channels through the sulcus. The surface of the sulcus will appear rough and is very easy to observe on the bone. Unlike Grade 1 preauricular sulcus, it would be difficult to overlook. Grade 4 typically looks like a deep channel in the bone.

For this research, each os coxae was individually graded. In addition, the maximum length and width of the sulcus was recorded, along with the sex of the individual. The data from the two collections were combined. The results showed a significant difference in the occurrence rates of the preauricular sulcus in males and females, demonstrating the value of this characteristic as a sexual indicator. A preauricular sulcus was present in 91.3% of females and not present in only 8.8%, while for males, preauricular sulci was only present in 39.5% of cases and are not present in the majority, 60.5%. The research also suggests that pregnancy and parturition does leave a mark on the sulcus. No Grades 3 or 4 sulci, which were suggested to be indicators of pregnancy and parturition, were found in males. They were only found in females that could have been parous, as 47.5% of females had Grade 3 and 8.8% had Grade 4. To verify this method, the proposed scoring system will be tested in the future on additional skeletal collections

According to the trend of modern forensic sciences, which are looking for valid and reliable methods, this study proposes a new scoring system to evaluate the morphology of the preauricular sulcus in relation to sex and possible parity status.

Sexual Dimorphism, Preauricular Sulcus, Pregnancy

A56 Bioarchaeological Investigations Discovered Intraindividual Bilateral Ossification Differences of the Medial Clavicle — Implications for Age Estimation of the Living

Fabian Kanz, PhD, Medical University of Vienna, Department of Forensic Medicine, Sensengasse 2, Vienna, YT 1090; Philipp Konermann, MD, Department of Forensic Medicine, Medical University of Vienna, Sensengasse 2, Vienna 1090; and Sandra Lösch, PhD, University of Bern, Institute of Forensic Medicine, Dept of Physical Anthropology, Sulgenauweg 40, Bern 3007, SWITZERLAND*

After attending this presentation, attendees will: (1) understand how bioarchaeological studies on human remains can support research in forensic anthropology; and, (2) be aware of possible ossification differences of the left and right clavicle and the implications for the procedure of age estimation of the living.

This presentation will impact the forensic science community in terms of knowledge and competence to modify the procedure of age estimation of the living by considering the development of the medial epiphysis of both clavicles, which will enhance the accuracy of the method.

Age determination in the living is put into practice both in criminal and asylum law. Within the standardized multifactorial examination by experts, the evaluation of the stage of ossification of the medial clavicle is of crucial importance. The complete fusion of the epiphysis is believed to be closely related to the time at which the age of legal majority is reached in many countries. In recent times, serious doubts arose concerning the assumption that bilateral ossification differences of the medial clavicle are negligible for age determination.[1] Asymmetric workload an individual is exposed to during his/her skeletal growth period might be responsible for differences in the ossification progress of the clavicles. A Medieval and an Early Modern Age population were chosen for investigation, assuming that workload conditions in historic times may better reflect the living conditions (hard manual labor) in countries from which the majority of the individuals subjected to age estimation procedures (asylum seeker and immigrants) in Austria and Switzerland originate.

The investigated individuals were collected during the archaeological excavation of a cemetery in St. Pölten in Lower Austria. Both clavicles of 70 females, 88 males, and 42 individuals with morphologically indeterminable sex were macroscopically investigated twice. Each clavicle was rated on the basis of the five-stage classification provided by Schmeling et al.[2] Definition of the five-stages (I–V) are: Stage I — non-ossified epiphysis; Stage II — discernible ossification center; Stage III — partial fusion; Stage IV — total fusion but epiphyseal scare still visible; and, Stage V — total fusion and epiphyseal scar no longer visible. Relative differences of the two clavicles in each ossification stage as well as inter-observer error as Overall Percentage Agreement (OPA) and Kappa value (κ) of the two independent observations were calculated. The χ^2 test was performed to investigate significant dissimilarities of side differences observed in the investigated ossification stages.

The distribution of the 200 investigated clavicle pairs was found to be nearly equal in the defined five stages and maximum side differences did not exceed one stage. In Stage I, 14.3% of the individuals showed side differences, 15.6% in Stage II, 50.0% in Stage III, 15.0% in Stage IV, and 13.5% in Stage V. For the individuals with known sex, the females (24.7%) tend to have stage differences more often than males (14.7%). The differences were found to be most pronounced in Stage III (females=61.5% and males=33.3%).

The inter-observer error turned out to be acceptable as evidenced by an OPA of 88.0% and the κ-value=0.83. The χ^2 test indicated significant differences (p=0.001, α=0.05) in the ossification stages only when Stage III was included. If Stage III was excluded, no significant differences between the Stages I, II, IV, and V could be found (p=0.995).

Since Stage III is most important for the decision of if an individual has already reached the age of legal majority or not, and the greatest bilateral differences were found in this stage. It is strictly recommended that both the left and right clavicle should be investigated during the age estimation procedure. In case of discrepancy, the clavicle expressing the ossification stage which promotes the interest of the investigated person should be favored in terms of "*in dubio pro minore*."

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Bioarchaeology, Medial Clavicle, Bilateral Ossification

A57 The Use of the Sustentaculum Tali in Estimating Sex

Christine Bailey, BA, University of Tennessee, Knoxville, 250 S Stadium Hall, Knoxville, TN 37996; Kristen A. Broehl, BA*, California State University, Chico, 400 W First Street, Chico, CA 95929; Amy Z. Mundorff, PhD, University of Tennessee, Dept of Anthropology, 250 S Stadium Hall, Knoxville, TN 37996; and Renee C. Kosalka, MA, 32 Thirty-Third Street, Toronto, ON M8W 3H1, CANADA*

After attending this presentation, attendees will better understand how the Sustentaculum Tali (ST) may be used in estimating sex in decomposed and skeletonized human remains. Attendees will also be introduced to new osteological measurements developed for this study.

This presentation will impact the forensic science community by providing an alternate method for metrically sexing skeletal remains. The sexually dimorphic parameters used in this research will be valuable in conjunction with other sexing techniques.

Sex estimation is a crucial component of the biological profile of unidentified skeletal remains; however, conventional methods of sex estimation are not always feasible when certain sexually dimorphic elements are missing or fragmented. Therefore, it is important to develop additional methods for sex estimation using non-traditional skeletal elements. Forensic practitioners have noted that calcanei are often recovered intact due to their density and protection within shoes and may, therefore, be a viable data source.[1,2] Previous research has also shown the calcaneus to be a sexually dimorphic skeletal element. These studies have considered the calcaneus as a whole but have not focused on dimensions of the ST in particular. Since the ST is a point of articulation between the calcaneus and talus, both sexually dimorphic bones, and existing sexually dimorphic measurements of the calcaneus encompass the ST, it is hypothesized that measurements of the ST will be useful in discriminating male and female calcanei.

The study was comprised of calcanei from 40 individuals, 20 male and 20 female, from the William M. Bass Donated Skeletal Collection at the University of Tennessee, Knoxville. Two researchers independently measured four parameters of the ST: Length (STL), Width (STW), Height (STH), and medial talar Facet length (STF). Measurements in this study were defined and each researcher measured the 40 calcanei three times to assess intra-observer error. A third researcher, uninvolved in developing the measurements, also measured the 40 calcanei to assess inter-observer error and the reliability of the definitions. Discriminant functions were derived using the Statistical Package for the Social Sciences (SPSS) v.21, and an additional sample of 28 calcanei was used to test the accuracy of the functions.

The results show sexual dimorphism for three measurements: STL (p=.000), STW (p=.050), and STF (p=.000). There was no significant difference between males and females for STH. Univariate discriminant analyses were run for the three sexually dimorphic dimensions, resulting in accuracies ranging from 60.0% to 77.5%. Direct discriminant function analyses were run using various combinations of the four variables, correctly classifying 72.5% to 75.0% of the sample. The test sample was classified using the discriminant functions derived from the original sample. The univariate functions accurately sexed 71.4% to 75.0% of the test sample, and the multivariate functions accurately sexed 75.0% to 85.7%.

The results of this study support the hypothesis that dimensions of the ST are useful for sex estimation. The measurements STL and STF had accuracies comparable to previous studies using the calcaneus. The results of this study are particularly important in cases where fragmented calcanei prevent the use of other measurements.

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Forensic Anthropology, Sex Estimation, Calcaneus

A58 The Roaming Arm: A Literal Outlier

Shana Ott, Metropolitan State Univ Denver, Chemistry Dept, PO Box 173362, Campus Box 52, Denver, CO 80217-3362; and Gary T. Scott, MA*, Metropolitan State University of Denver, Dept of Anthropology and Sociology, 1201 5th Street, Campus Box 28, Denver, CO 80204*

After attending this presentation, attendees will better understand animal scattering of human remains, distances of transport, and how this may affect search and recovery planning.

This presentation will impact the forensic science community by presenting a case study of a suspected animal transport of a human arm for more than one mile, which exceeds previously documented scatter distances.

Per Haglund, regarding the taphonomy of human remains, dog and coyote scavenging results in consumption, disarticulation, modification of bone, and scattering of remains.[1] Having knowledge of the fauna, flora, seasonality, clothing, postmortem interval, topography, and environment could all impact the distance that human remains are scattered.[2-4] This information is critically important when disarticulated human remains are located in an outdoor setting and recovery is required. In forensic contexts, following the initial discovery and reporting of human remains, it becomes the responsibility and jurisdiction of law enforcement, coroners, and medical examiners to search for any missing remains. Search parameters often are influenced by budgetary constraints; therefore, data on scatter patterns and distances will assist in pre-search planning.

On June 2, 2015, a group of hikers discovered a desiccated, articulated, skeletonized left arm (scapula, clavicle, humerus, radius, ulna, carpals, metacarpals, and most phalanges). These remains were reported to and recovered by the Park County Coroner and Park County Sheriff's Department. A subsequent search in the area by law enforcement, Colorado Forensic Canines, Search and Rescue Dogs of Colorado, Park County Search and Rescue, and Metropolitan State University of Denver Human Identification Laboratory personnel did not result in finding additional human remains.

On July 8, 2015, a hiker went off trail in Park County, CO, and discovered a desiccated, mostly articulated human skeleton. The left arm was missing. The body was exposed and on the ground surface. Underwear, jeans, socks, and boots, remained on the body; a shirt and jacket were nearby.

The body had a rope around the neck and presented as a suicide; body positioning and vegetation discoloration were all consistent in indicating that the remains were at their original deposition site. While it was suspected that the body and the previously discovered arm were from a single individual, the cases were processed separately. Subsequently, the body was positively identified as those of an 18-year-old male. The individual had been reported missing on October 22, 2014, his car was discovered at a Trailhead in Park County, CO, on October 24, 2014, and a suicide note was found inside the car; this initiated a search, but the individual was not found.

Subsequently, the arm was matched to the body (no overlapping remains, the bones were consistent in size, morphology, and degree of decomposition) and both displayed minimal carnivore gnawing and chewing.

When comparing the Global Positioning Coordinates of the body and left arm, it was found that they were separated by a straight-line distance of more than one mile (1,900 meters), which significantly exceeds the documented distances within the literature. Haglund states that, following scavenging, most skeletal elements are recovered within a 100-meter radius of where the body was found.[1] Haglund and others have documented maximum transport of remains at distances of 402, 291, 200, and fewer meters.[5-7]

A number of factors can play a role in the scatter of human remains including mammals, plants, topography, climate, clothing, and postmortem interval, among others. The remains were located 2,500 meters (8,200 feet) above sea level, in steep terrain with a mix of conifer trees, shrubs, and boulders, exposed to temperatures ranging from -23°C to 24°C (-10°F to 75° F) and monthly snowfall amounts ranged from 8-86 centimeters (3-34 inches) during the months of November 2014 through May 2015. It is important to document these variables to assist with subsequent search planning and briefings.

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Scavenging, Scattered Remains, Searches

A59 No Fly Zone: Decomposition in the Absence of Insects

Michael S. Woolf, BS, Virginia Commonwealth University, 6732 Hopton Court, Richmond, VA 23226; Tal Simmons, PhD*, Virginia Commonwealth University, Dept of Forensic Science, 1015 Floyd Avenue, Richmond, VA 23284; and Baneshwar Singh, PhD, Virginia Commonwealth University, Dept of Forensic Science, 1015 Floyd Avenue, Rm 2015, Richmond, VA 23284*

After attending this presentation, attendees will better understand how insects drive the decomposition process when all other variables are held constant.

This presentation will impact the forensic science community by providing results from a controlled experiment which compared decomposition rate and pattern between insect-access and insect-exclusion groups of pigs. These results are compared to previously published work using different animal models.

Although Accumulated Degree Days (ADD) provide a framework for comparing decomposition in disparate regions, temperature is not the sole impetus of decomposition; as many have noted, insects are one of the primary drivers of biomass removal leading to skeletonization. Previously published work compared the process of decomposition in the presence and absence of insects using a combination of previously published, experimental, and retrospective data; the results indicated that the absence of insects (whether achieved through deposition indoors, in burial, or in water) slowed the rate of decomposition in the same manner and regardless of weight.[1]

In this experiment, 12 pigs (*Sus scrofa*) weighing 22kg-32kg, killed by a .22 caliber bullet to the head, were placed in an open field at the Virginia State Police Training Center in Hanover, VA, on June 10, 2015. All pigs were placed individually in scavenger-proof wire cages, 10m apart. Six cages were also enclosed within an outer tent constructed of Polyvinyl Chloride (PVC) pipe overlaid with fine mesh netting to exclude insects. These tents were weighted with sandbags along the bottom edge and could be entered through a zippered opening in one side, the bottom of which was covered with a flap and weighted with another sandbag. To mimic the partial shade provided by the insect exclusion nets, shade cloth was placed on the top of each insect access cage. Temperature onsite was recorded hourly by a datalogger attached to one of the cages. Pigs were observed daily for the first ten days (to 296 ADD), on alternate days for the succeeding two weeks (to 575 ADD), then once a week for two more weeks (to 903 ADD) when the insect-accessed pigs had reached Total Body Score (TBS) of 26-30 on the revised 32-point scale, indicating >50% bone exposure, with mummification and some areas exhibiting greasy bone only.[2] Insects were collected and identified at each visit.

Adult flies from family Calliphoridae (*Phormia regina*, *Lucillia spp.*, and *Cochliomyia macellaria*) and beetles from families Silphidae (*Necrophila americana*, *Necrodes surinamensis*, and *Oiceoptoma novaboracense*) and Staphylinidae (*Creophilus maxillosu*s) were observed in large numbers during early decomposition stages (fresh and bloat). Egg masses and larvae were also noted within 24hrs (27 ADD). In addition to those aforementioned species, beetles from family Cleridae (*Necrobia rufipes*) were noted during later decomposition stages (active decay, advanced decay, and dry). Overall, *Phormia regina* and *Creophilus maxillosus* were the most prevalent insect species throughout this study. Peak maggot masses migration occurred between days 7-8 (237 to 267 ADD).

Preliminary linear regression indicates a strong, positive linear relationship between ADD and TBS for the exclusion and access groups with R^2 values of 0.9118 and 0.9118, respectively. Slopes and intercepts for both estimated regression lines are different. The equation for the insect exclusion group is TBS=-15.6760+11.0134 ($\log_{10}$ADD) while the equation for insect access group is TBS=-30.9506+20.9872 ($\log_{10}$ADD). Analysis of Variance (ANOVA) ($p \leq$6.193e-13) and Analysis of Covariance (ANCOVA) ($p \leq$2.2e-16) indicate that the difference in group means and the effect of ADD on TBS are statistically significant. Linear mixed-effects modeling will also be used to assess TBS as the response variable and group assignment (insect access or exclusion) as a random variable.

In conclusion, this study provides further evidence that necrotizing insects function as primary colonizers that accelerate decomposition with a strong, positive linear relationship to ADD. Additionally, this study provides much-needed geographical data for the development of model-based methods for estimation of human Postmortem Interval (PMI).[2]

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Taphonomy, Insects, Decomposition

A60 White-Tailed Deer as a Taphonomic Agent: Photographic Documentation of White-Tailed Deer Gnawing on Human Bone

Daniel J. Wescott, PhD, Texas State University, Dept of Anthropology, 601 University Drive, San Marcos, TX 78666-4684; Lauren Alyssa Meckel, BS*, Texas State University, 1509 Marlton Street, San Marcos, TX 78666; Chloe P. McDaneld, 125 Amberwood Cove, Kyle, TX 78640; Michelle D. Hamilton, PhD, Texas State University, Dept of Anthropology, 601 University Drive, San Marcos, TX 78666; Sophia Mavroudas, MA, Texas State University, 601 University Drive, ELA 232, San Marcos, TX 78666; and Kate Spradley, PhD, Texas State University, Dept of Anthropology, 601 University Drive, San Marcos, TX 78666*

After attending this presentation, attendees will better understand the damage caused to bone by white-tailed deer gnawing.

This presentation will impact the forensic science community by providing photographic documentation of white-tailed deer gnawing on human bone and therefore acting as a taphonomic agent.

While most forensic anthropologists and taphonomists are aware that carnivores and rodents gnaw on and consume human bones, the fact that cervids and other ruminant species also chew on bone is not as widely recognized. Cervids (e.g., deer, elk, moose) gnawing on bone has been reported in the taphonomic and zooarchaeological literature, but there is no known report of cervids altering human remains.[1-4] This study reports on the first known documented case of white-tailed deer gnawing on human skeletal remains and discusses distinguishing features of ungulate gnawing and the reasons for this behavior.

The Forensic Anthropology Center at Texas State University (FACTS) accepts human donations for taphonomic research. As part of an ongoing research project to document scavenging activities on naturally decomposing human remains, a motion-sensitive camera was place approximately 4.5 meters from an uncaged human body.[5] The donated body was placed in a small wooded area at the Forensic Anthropology Research Facility (FARF) in July 2014. The body was initially scavenged by vultures that removed much of the soft tissue, leaving a mostly articulated skeleton and large pieces of desiccated skin. At approximately 190 days postmortem, a motion-sensitive cameras captured multiple images of a young deer with a human rib bone in its mouth on two different occasions.

Upon discovery of the photographs, the skeletal remains were investigated more closely. The two ribs gnawed by the deer had been disarticulated from the vertebral column and moved less than one meter from the articulated trunk. The ribs exhibited splintering of the sternal ends that has been characterized as "forking," but no obvious signs of tooth depressions, punctures, or grooves. The forking is characteristic of ungulate damage and was caused by the deer holding the sternal end in its mouth parallel to the tooth row and rubbing its teeth against the bone.[1]

Deer and other cervids most likely gnaw on or consume bone to obtain phosphorus, calcium, and other minerals absent from their vegetarian diet, especially in the winter, and prefer relatively fresh bone. In addition to forking, cervid damage to bone can include tooth impressions, grooves, and punctures, which are also common in bone gnawed on by carnivores and rodents; however, the damage caused by deer and other ruminant species can be distinguished from modifications caused carnivores and rodents.

While cervids do not greatly contribute to the scavenging guild, they should not be overlooked as a possible taphonomic agent in the modification of human remains in medicolegal death investigations. In regions with large cervid populations, forensic scientists should be aware of the potential damage that can be caused to bone by cervid species.

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Osteophagia, Taphonomy, Bone Modification

A61 Examining the Persistence of Human DNA in Soil During Cadaver Decomposition

Alexandra L. Emmons, MA, University of Tennessee, 2831 Island Home Avenue, Knoxville, TN 37920; Jennifer DeBruyn, PhD; Amy Z. Mundorff, PhD, University of Tennessee, Dept of Anthropology, 250 S Stadium Hall, Knoxville, TN 37996; Kelly L. Cobaugh, MS, University of Tennessee, 2506 E.J. Chapman Drive, Knoxville, TN 37996; and Graciela S. Cabana, PhD, University of Tennessee, 250 S Stadium Hall, Knoxville, TN 37996-0720*

After attending this presentation, attendees will gain a more thorough understanding of the persistence of human DNA in the soil during human cadaveric decomposition.

This presentation will impact the forensic science community by complementing existing information concerning taphonomic changes in the soil environment during human decomposition. The results of this project will progress the understanding of the persistence of human DNA in soil, thereby expanding upon the current understanding of the interplay between chemical, physical, and biological processes occurring in the soil in concert with human decomposition.

The majority of experimental work involving human decomposition has focused on aboveground processes, ignoring the potential impact imposed on the underlying soil. Though recent decades have seen a marked increase in research of this type, including the fate of certain cadaveric biological correlates once they enter the soil, the fate of another important biological correlate in grave soil — human DNA — has been relatively understudied.[1-10] This study sought to redress this gap in existing knowledge by assessing the persistence (i.e., presence or absence) of human nuclear and mitochondrial DNA (mtDNA) and evaluating the quantity of recovered DNA from soil over the course of decomposition of four human cadavers placed at the University of Tennessee's Anthropological Research Facility.

To test hypotheses that both human nuclear and mtDNA would be recoverable from the soil environment and that the quantity of DNA would be greatest during active and advanced decay stages of decomposition, samples were assessed using end-point and real-time quantitative PCR (qPCR). Cadaver DNA from soil samples was verified by comparing sequences from the human mtDNA control region (HVI and HVII) between cadaver blood samples and a subset of soil samples taken from below each cadaver following the initiation of decomposition.

Human nuclear DNA was largely unrecoverable from the soil throughout decomposition, while cadaver mitochondrial DNA was detectable throughout all decomposition stages. MtDNA copy number increased as decomposition progressed, peaked during active decay (Max. Value=$1.9X10^6$ copies gdw^{-1}), and declined throughout the remainder of decomposition, reaching a minimum value of $1.4X10^4$ copies gdw^{-1}. When tested against additional variables including time (measured in Cumulative Degree Hours (CDH)) and soil chemistry, mtDNA copy number showed a positive correlation with CDH (r_s=0.420, p=0.041), Total Organic Carbon (TOC) (r_s=0.418, p=0.042), and Total extractable Nitrogen (TN) (r_s=0.569, p=0.004).

In conclusion, human mtDNA can be recovered from soil and is of a high enough quality to be used for exclusionary purposes during identification efforts.

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Soil Taphonomy, Human DNA, Identification

A62 Using the Geographic Information System (GIS) to Distinguish Between Human and Non-Human Cranial Bone Fragments

Brigida Corrieri, MSc, Cranfield University, Cranfield Forensic Institute, Defence Academy of the United Kingdom, Shrivenham, Oxfordshire SN6 8LA, UNITED KINGDOM; Nicholas Márquez-Grant, PhD*, Cranfield University, Cranfield Forensic Institute, Defence Academy of the United Kingdom, Shrivenham SN6 8LA, UNITED KINGDOM; Jessica Bolton, MSc, Cranfield University, Cranfield Forensic Institute, Defence Academy of the United Kingdom, Shrivenham SN6 8LA, UNITED KINGDOM; and Roland Wessling, MSc, Cranfield Univeristy, Cranfield Forensic Institute, Shrivenham, Oxfordshire SN6 8LA, UNITED KINGDOM*

After attending this presentation, attendees will be aware of the utility of GIS in the distinction between human and non-human cranial fragments. The method presented shows an innovative application of the geographical system, which was used to measure the sutures pattern and the bone curvature of human and non-human skulls, with the goal of creating a database that can be used for a quick identification of small cranial fragments.

This presentation will impact the forensic science community by demonstrating that GIS, a system usually used to manage geographic data, can be a useful tool for the differentiation between human and non-human fragmented cranial bones, with the advantage of being a cheap and non-destructive method that can speed up the identification process.

Cranial bone fragments can be found isolated in forensic contexts, such as fatal fires and mass disasters. In some instances, when bone fragments are present, police will call upon the anthropologist to understand if they are human or not. Whether the bone is confidently assigned as human or not will have repercussions for the investigation and will affect cost and time spent in the investigative process. Indeed, some small cranial bone fragments can present a real challenge for anthropologists and as many methods as possible must be used in order to establish their human or non-human origin.

Human and non-human crania, both juvenile and adult, were employed for the GIS measurements. The animal species chosen for the study were fox, cattle, and sheep. The skulls of these animals may be problematic if found fragmented, because they share some characteristics with human ones. For example, the parietal bone of the fox and calf has a curvature similar to that of humans, and fox and sheep cranial sutures may resemble some of the human skull sutures. Furthermore, these animals were selected because they are commonly found in the United Kingdom.

This presentation details the results of the analyses made on crania with GIS, demonstrating its potential in this aspect of forensic anthropology. Selected cranial sutures of all the skulls were mapped, and the curvature of specific cranial bones was measured. The measurements were then entered into a database. When cranial bone fragments are found, the sutures (if present) can be scanned and compared to the ones present in the database in order to ascertain if they are of human or non-human origin; the same process can be applied when measuring and comparing the bone curvature.

Though variations can occur, particularly related to age and/or pathological conditions, cranial sutures and curvature tend to follow a specific pattern in both human and non-human skulls. Therefore, they can be measured and compared with GIS, which was demonstrated to be a useful, fast, and non-destructive tool for the distinction between human and non-human cranial bone fragments in forensic anthropology.

Bone Fragments, GIS, Forensic Anthropology

A63 Application of Stable Isotope Forensics for Predicting Region-of-Origin of Unidentified Border Crossers Found Deceased in the United States

Eric J. Bartelink, PhD, California State University, Chico, Deparment of Anthropology, Butte 311, 400 W First Street, Chico, CA 95929-0400; Heather L. MacInnes, BS, 1775 E 8th Street, Chico, CA 95928; Julia R. Prince, BA, 1775 E 8th Street, Chico, CA 95928; Amy T. MacKinnon, BA, 400 W 1st Street, Chico, CA 95929; Lesley A. Chesson, MS, IsoForensics, Inc, 421 Wakara Way, Ste 100, Salt Lake City, UT 84108; Brett J. Tipple, PhD, IsoForensics, Inc, 421 Wakara Way, Ste 100, Salt Lake City, UT 84108; Krista E. Latham, PhD, University of Indianapolis, Biology Dept, 1400 E Hanna Avenue, Indianapolis, IN 46227; and Gregory E. Berg, PhD, DPAA Identification Laboratory, 310 Worchester Avenue, Joint Base Pearl Harbor-Hickam, HI 96853-5530*

After attending this presentation, attendees will understand the application of stable isotope analysis in predicting the region-of-origin of unidentified border crossers found deceased within the United States. Attendees will better understand the applications and limitations of stable isotope analysis as an investigative tool for forensic scientists in identifying foreign nationals.

The development of this form of forensic analysis will impact the forensic science community by demonstrating how stable isotope analysis can aid the repatriation process for unidentified border crossers from Latin America.

Over the past two decades, there has been a steady increase in the number of deaths of Unidentified Border Crossers (UBCs) along the United States-Mexico border. Since 1999, there have been more than 6,000 deaths in the United States border states (especially Arizona and Texas), representing Mexican, Central American, and South American nationals. The large volume of UBC casework has created an unprecedented human identification challenge, especially given the lack of personal documentation, antemortem records, and DNA family reference samples for these individuals.

Recently, a large-scale effort has been mounted to identify deceased UBCs recovered from Brooks County, TX. This area is along a major migration route from Mexico into Texas and has experienced a large number of UBC deaths in recent years. In 2013, Baylor University and the University of Indianapolis began the process of exhuming UBCs from this region to aid in personal identification efforts. Current efforts toward identification have focused on DNA, craniometrics, personal effects, and the use of missing persons databases.

Stable Isotope Analysis (SIA) can provide another investigative tool to aid in the identification effort of UBCs. Recently, SIA has been successfully used to provenance human remains from past wars and conflicts, as well as unidentified human remains cases from local jurisdictions. Stable isotope ratios measured in bones, teeth, and hair can provide a record of a person's dietary preferences, recent travel history, and childhood residence. Dietary information gleaned from stable carbon and nitrogen isotope values of bone collagen and bioapatite, tooth enamel bioapatite, and hair keratin provide useful information on an individual's food consumption practices during life. More importantly, stable oxygen and strontium isotopes in human tissues can be used to predict a region-of-origin. Because stable oxygen isotopes of water vary based on environmental factors, isotope ratios measured in bones and teeth reflect the local water source imbibed at the time of tissue formation. Strontium isotopes reflect geological age of a region's underlying bedrock and are incorporated into humans who consume plant and animal resources from the local landscape. When combined, these isotopes provide a powerful "geolocation" tool (i.e., an "isoscape") for predicting an individual's region-of-origin or travel history.

The goal of this study is to present stable isotope results and isoscape maps for a subset of UBCs (n=13) recovered from Brooks County, TX. Human bone and tooth samples were prepared for mass spectrometry, including stable carbon and nitrogen isotopes of bone collagen, stable carbon and oxygen isotopes of enamel bioapatite, and strontium isotopes of enamel.

Mean bone collagen $\delta^{13}C$ is -14.1‰ (±1.9, 1 Standard Deviation (SD); range=6.5) and mean $\delta^{15}N$ is 10.3‰ (±1.1, 1 SD; range=4.2). Tooth enamel bioapatite, which reflects childhood diet, is even more variable, with a mean $\delta^{13}C$ value of -6.8‰ (±2.4, 1 SD; range=7.6). As expected for individuals of Latin American origin, these mean values are consistent with a diet that emphasized C_4-based resources (e.g., corn products). The extent of dietary heterogeneity suggests that these individuals may be from different regions within Latin America; however, two individuals have especially low $\delta^{13}C$ bone and tooth bioapatite values and somewhat high $\delta^{15}N$ values, suggesting a diet more focused on C_3-based resources and higher trophic level protein sources.

For enamel bioapatite, mean $\delta^{18}O$ is -5.0‰ (±1.7, 1 SD; range=6.0). Isoscape predictions based on precipitation water maps are consistent with several Latin American countries for the majority of the sample. In at least one case, the $\delta^{18}O$ value is only consistent with an origin within the United States and in another case, only within the northeastern coast of South America. Stable isotope analysis can provide a useful investigative tool to aid in the identification effort of UBCs. The addition of the strontium isotope data should aid in narrowing down a more specific region.

Forensic Anthropology, Identification, Human

A64 What Level of Biogeographical Information Is Available From ^{18}O and ^{13}C Signatures in Late-Erupting Molars of Modern Humans?

Anastasia Holobinko, MS, Southern Illinois University, Dept of Anthropology, MC-4502, Carbondale, IL 62901; Wolfram Meier-Augenstein, PhD, Robert Gordon University, School of Pharmacy & Life Sciences, Sir Ian Wood Building, Garthdee Road, Aberdeen AB10 7GJ, UNITED KINGDOM; Helen F. Kemp, PhD, OEA Laboratories Ltd, Kelly Bray, Callington PL17 8EX, UNITED KINGDOM; Susan M. Ford, Southern Illinois University, Dept of Anthropology, MC4502, Carbondale, IL 62901; and Philip Turk, PhD, Colorado State University, Dept of Statistics, Rm 200, Statistics Bldg, Fort Collins, CO 80523*

After attending this presentation, attendees will better understand the forensic application of stable isotope analysis as it pertains to determinations of human provenance and the potential interpretive difficulties associated with analyses of enamel carbonate isotopic data.

This presentation will impact the forensic science community by illustrating the complexities associated with inferring geographic origins from isotopic data obtained from living individuals with self-reported dietary preference and residential history.

The goal of this presentation is to examine intra- and inter-tooth isotopic variability in the abundance of ^{18}O and ^{13}C in third molar enamel carbonate from individuals with self-reported dietary preferences and residential history and to explore this variability as an indicator of intra-individual variability.

Stable isotope analysis of biogenic tissues such as tooth enamel and bone mineral has become a well□recognized and increasingly important method for determining the provenance of human remains. Both ^{18}O and ^{2}H stable isotope signatures are well-established proxies as environmental indicators of climate (temperature) and source water and are therefore considered reliable indicators of geographic life trajectories of animals and humans.[1,2] Similarly, ^{13}C and ^{15}N abundance data have distinguished dietary preferences in ancient human populations, and have been used to qualify $_{2}H$ and ^{18}O geolocational data that may be consistent with more than one location.[3]

Third molar tooth enamel was sampled from ten living volunteers undergoing routine tooth extractions at Canadian dental clinics. Patients provided detailed residential history and answered questions pertaining to dietary preferences (e.g., vegetarian) prior to donating all four third molars. Enamel was drilled from the crown of two third molars from each subject, chemically cleaned, and subjected to an acid digest before being analyzed for their ^{18}O and ^{13}C composition using Isotope Ratio Mass Spectrometry (IRMS).

The pooled mean enamel carbonate $\delta^{13}C_{VPDB}$ value for all samples was suggestive of a persistent C_4 plant dietary influence at the time the sampled tooth enamel was forming. This is consistent with self-reported dietary intake information and residential history and with what is known about the typical North American diet.[4-6]

The pooled mean $\delta\ ^{18}O_{VSMOW}$ value for enamel carbonate from all samples was 24.39 ‰. Although subject variation was significant, neither diet nor sex significantly influenced the oxygen isotope data. Following conversion of $\delta\ ^{18}O_{Carbonate}$ values to $\delta\ ^{18}O_{Phosphate}$ values, drinking water $\delta\ ^{18}O$ values were calculated and compared to their corresponding regional estimated annual average $\delta\ ^{18}O$ values in precipitation retrieved from the Online Isotopes in Precipitation Calculator (OIPC).[7-9] No statistically significant correlations were evident between drinking water $\delta\ ^{18}O$ values and $\delta\ ^{18}O_{OIPC}$ values.

The overall lack of strong linear relationships between calculated drinking water $\delta\ ^{18}O$ values and precipitation $\delta\ ^{18}O$ values in this particular dataset illustrates the importance of considering site-specific isotopic complexities and using multi-isotope data obtained from multiple tissues when investigating the geographic origins of humans in an archaeological or forensic context. It is not possible to quantify intra-individual isotopic variability without sampling from larger populations and controlling for as many variables as possible. The construction of a database containing isotopic data obtained from a variety of environmental, human, and faunal tissue samples, and the application of such data to individual cases in which geographic origins are desired, is recommended.

While standardization of analytical methodology is critical to appropriate interpretations of the data, stable isotope profiling is not a stand-alone method and should be used in conjunction with other lines of evidence in determinations of human provenance.

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Stable, Provenance, Identification

A65 Dental Non-Metric Analysis as an Aid to Undocumented Border Crossers (UBCs) Region-of-Origin Estimation

Rebecca L. George, MA, 9455 Sky Vista Parkway, Apt 10E, Reno, NV 89506; and Jorge Gómez-Valdés, PhD, Universidad Nacional Autónoma de México, Dept of Anatomy, Av. Universidad 3000, Mexico DF 04510, MEXICO*

After attending this presentation, attendees will better understand the application of dental non-metric analyses to the process of identifying deceased UBCs, as they help narrow an estimated region-of-origin for these individuals.

This presentation will impact the forensic science community by demonstrating the utility of dental non-metrics as a part of ancestry estimation within the biological profile, particularly in reference to how this methodology can contribute to the ongoing research seeking to increase UBC's identification rates.

Dental non-metric analyses have been used for more than 100 years in ancestry studies and were standardized through the establishment of the Arizona State University Dental Anthropology System (AUDAS) by Turner et al. in 1991.[1,2] Per Birkby et al., though, only two dental non-metric traits are utilized by the Pima County Office of the Medical Examiner (PCOME) in the UBC identification process.[3] This is not surprising, given that much of the recent UBC literature has focused on cranial and postcranial studies. Non-metric cranial studies primarily differentiate American Blacks and Whites from known UBC samples.[4-5] The metric postcranial study, though, found a need for population-specific formulas when working with known UBC individuals, as not all of the decedents originate from the same geographical location and, therefore, cannot be pooled under one ancestry umbrella term.[6] A craniometric study from within Mexico highlights this need for population-specific formulas as regional skeletal variation exists.[7] There is also a dental non-metric study that focuses on American Hispanic differentiation that highlights these same results from the aforementioned studies.[8] Edgar discussed the difficulty in distinguishing American Hispanic groups from one another when compared in a large study sample with American Whites and Blacks. The results of this study indicate that there may be potential for dental non-metric analyses when population-specific trait suites are established.

To explore the utility of dental non-metric analyses as they pertain to UBC individuals, samples were selected from Albuquerque, NM, Mexico City, Mexico, Zimapán, Hidalgo, Phoenix, AZ, and from unidentified UBC individuals housed at the PCOME, as well individuals from the Sacred Heart Burial Park from Falfurrias, TX, housed at Texas State University. Seventy-five American Southwest Hispanic (ASH) individuals were included in analysis, as well as 90 Mexican individuals and 33 unidentified UBC individuals; UBC individuals were either pooled or separated for certain analyses based on the small sample size of this group. These region-of-origin groups were selected based on the prevalence of UBC individuals that have been previously identified as Mexican at the PCOME and the need to determine any potential differences between ASH and foreign-born Hispanics. The dental non-metric data were collected according to the Arizona State University Dental Anthropology System (ASUDAS) standards and dichotomized for analyses.

A series of Pearson's chi-square and Fisher's exact tests to compare dental non-metric trait prevalence between each of the three region-of-origin groups demonstrated a lack of M_2 protostylid in the ASH sample (15.7%), while it was present in the Mexican (67.8%) and UBC (52.4%) groups in higher percentages of the total samples. The Lower Canine Distal Ridge (LCDR) (82.4%) and M_1 cusp 7 (53.8%) were more prevalent in the UBC group than in the ASH (49.1% and 25.0%, respectively) and the Mexican groups (42.1% and 25.0%, respectively). Furthermore, Mean Measure of Divergence (MMD) indicated that the ASH group differed significantly from the Mexican group (0.106) and the ASH group differed significantly from the UBC group from the Sacred Heart Burial Park (0.103).

These results indicate there are regional differences in dental non-metric trait prevalence among the ASH, Mexicans, and some of the unidentified UBCs included in these analyses. While there are limitations of this current research due to the samples selected for study, these are promising indications of the utility of dental non-metrics in UBC studies, nonetheless. Additionally, when placed in the framework of existing UBC literature, this research supports the assertions that population-specific trait suites and formulas are necessary to further the identification process of UBC individuals.

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Dental Non-Metrics, Ancestry Estimation, UBC

A66 Comparative Study of Human and Non-Human Long Bones by Anatomical and Radiological Methods

Piyush Sharma, MD, All India Institute of Medical Sciences, Rm No-302, Dept of Forensic Medicine, New Delhi, Delhi 110029, INDIA; and Tabin Millo, MD, Department of Forensic Medicine, AIIMS, MMRDH, New Delhi 110029, INDIA*

After attending this presentation, attendees will better understand how human and non-human long bones differ when compared using some specific anatomic and radiological measurements, especially when only the mid-shaft of the bone is available, which is very difficult to identify.

This presentation will impact the forensic science community by providing new parameters and statically significant results from a comparative cross-sectional study in an area in which limited research is performed and will add to research being performed in forensic anthropology and forensic pathology by widening the prevalent view of the differences as well as use of new parameters for differentiating the mid-shaft region of long bones of human and non-human origin.

With skeletal remains, the first step is to determine whether or not the object in question is actually bone, and if so, then whether or not it is human. Many organic and inorganic materials can mimic bone (e.g., wood, stones, etc.)[1] When bones are incomplete or fragmentary, problems escalate rapidly. Cylindrical segments of the central shaft have little in the way of distinguishing features, apart from size. Burnt bone fragments offer similar problems due to heat distortion and shrinkage.[2] False samples of bones could be incorporated and claimed to be of human origin. Such cases lead to medicolegal complications such as whether the bones found could be linked to murder.

In this study, 30 human long bones of upper and lower limbs and 30 corresponding bones of *Capra aegagrus hircus* (goat) and *Ovis aries* (sheep) were used. This study was conducted at the Department of Forensic Medicine, at a tertiary care hospital in New Delhi after receiving ethical clearance. The continuous variables were compared in two groups by independent *t*-test and Wilcoxon rank sum test. Categorical variables were compared in two groups by using chi-square test and Fischer's exact test. The *p* value <0.05 was taken to be statistically significant.

The comparison of cortical thickness and ratio of cortical thickness to total diameter was statically significant when the group comparison was performed, which agrees with Croker et al.[3] But, when individual human bones were compared with non-human bones as a group, femur (p=0.39), fibula (p=0.45), humerus (p=0.57), and radius and ulna (p=0.34) showed no significant results for cortical thickness to total diameter ratio. When compared to the non-human counterpart, tibia (p=0.5) and fibula (p=0.22) showed no significant results. The mean ± Standard Deviation (SD) values for cortical thickness in this study for the human group was 5.36 ± 2.40 and for the non-human group was 2.89 ± 0.87 (p<0.05). In this study, the cortical thickness for the tibia in the mid-diaphyseal region had a median value of 8.87mm, similar to the findings obtained by Croker.[4] When compared with animal counterparts, cortical thickness to total diameter ratio was statically significant for the radius (p<0.05) and ulna (p<0.05) but was non-significant for the femur (p=0.39), tibia (p=0.5), humerus (p=0.57), and fibula (p=0.45). The median for human femur bones was 0.271 and for non-human bones was 0.198 (p=0.39). In this study, the length of the long bones and the presence of nutrient foramen in mid diaphyseal region doesn't differentiate between the two groups, which correlates with the studies by Chatrapathi and Shamsunder.5,6 For all long bones except the femur, a sharp border delineating the cortex and medulla in X-rays was present (p<0.05). For the fibula, humerus, radius, and ulna, parameters such as circumference at mid diaphyseal region, cortical thickness, antero-posterior diameter, presence of diaphyseal trabeculae, and cortical thickness to total diameter ratio were found to be insignificant.

In conclusion, this study attempts to shed light upon a gray and often neglected area — anthropology. This presentation will greatly impact criminologists and anthropologists, as it is a common scenario encountered in routine practice. This presentation will also help establish a baseline determinant for human bone differentiation, which will aid further studies and yield a fruitful medicolegal outcome. With the advent of modern scientific tests, human anthropometry has become a mere platitude of sorts; this study attempts to reach back to the grassroots of anthropometry and usher in a new scientifically backed method of human bone identification and differentiation.

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Anthropometry, Skeletal Remains, Animal Bones

A67 Manipulation and Analysis of Virtual Bones: A Novel Method of Sex Estimation From the Mandible

Alice J. Butcher, BSc, Cranfield University, Cranfield Forensic Institute, Shrivenham, Oxfordshire SN6 8LA, UNITED KINGDOM; Roland Wessling, MSc, Cranfield Univeristy, Cranfield Forensic Institute, Shrivenham, Oxfordshire SN6 8LA, UNITED KINGDOM; Jessica Bolton, MSc, Cranfield University, Cranfield Forensic Institute, Defence Academy of the United Kingdom, Shrivenham SN6 8LA, UNITED KINGDOM; and Jelana Bekvalac, MSc, Museum of London, 150 London Wall, London EC2Y 5HN, UNITED KINGDOM*

After attending this presentation, attendees will better understand the use of 3D laser scanning to create virtual models of human skeletal remains and how these models can be automatically manipulated and analyzed in novel ways to build up a biological profile. The principles involved in these processes are exemplified through a feasibility study on automated sex estimation using topographical analysis of the mandible.

This presentation will impact the forensic science community by highlighting the capabilities and advantages of automated, computer-based anthropological analysis and by introducing a new avenue for sex estimation of skeletonized human remains.

Analysis of virtual osteological material is an emerging field in physical anthropology, conferring advantages over traditional methods in areas such as reproducibility, data handling, and retention, as well as range of possible measurements and unique analyses available. This study, which is part of the Virtual Skeletal Analysis (ViSA) Project, attempted to apply these principles to the mandible with regard to sex estimation.

Thirty-four complete, non-pathological human mandibles from the St. Bride's Church crypt collection in London were digitized using hand-held laser topography. Using a tailor-made R[1] package based on the geometric properties of the bones, each resulting point cloud was manipulated into a standardized orientation. The exterior surfaces of the posterior ramus borders and mandibular angles were separated from the main body and re-oriented for further analysis.

A triangular mesh network was formed over the point clouds representing the posterior rami and mandibular angles and these were analyzed for mean slope, mean aspect, and size (2D area, 3D surface area, and valley volume) using Geographic Information Systems (GIS) software; an uncommon approach in anthropological analysis that has never previously applied to the mandible. This software treats the bone surface as a miniaturized landscape, providing a unique and quantifiable method of analyzing bone morphology.

It was found that both the posterior rami and mandibular angles were sexually dimorphic in terms of size, either bilaterally or unilaterally (particularly on the right side), and in the mean slope of the left posterior ramus. Preliminary demarcation points for the estimation of sex were created and tested for each sexually dimorphic analysis. The 2D area of the right posterior ramus and valley volume of the right mandibular angle were each independently able to correctly sex over 80% of the sample.

This particular methodology must be tested on further samples to confirm and refine its findings; however, the study successfully revealed a largely unexplored territory of anthropological analysis, with great capacity to expand. The study especially exposed the dearth of specialized software for the virtual analysis of skeletal material, despite the considerable advantages and potential for the field.

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Virtual, Skeletal, Analysis

A68 Calcium and Phosphorus Detection Using Benchtop vs. Hand-Held X-Ray Fluorescence (XRF) Spectrometers

Aaron R. Kuzel, BS, Lincoln Memorial University - DeBusk, College of Osteopathic Medicine, 6965 Cumberland Gap Parkway, Harrogate, TN 37752; Angi M. Christensen, PhD, FBI Laboratory, 2501 Investigation Parkway, Quantico, VA 22135; and Susan M. Marvin, PhD, FBI Laboratory, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, attendees will be familiar with the different detection levels of calcium and phosphorus when using hand-held and benchtop XRF spectrometers.

This presentation will impact the forensic science community by confirming that XRF-generated Calcium to Phosphorus (Ca/P) ratios using either hand-held or benchtop XRF devices are a valid criteria for distinguishing between different material sources and by highlighting the need for users to evaluate Ca/P ratios in reference to those made under similar analytical conditions.

The analysis of skeletal remains (or potential skeletal remains) for chemical and elemental properties is becoming increasingly common in forensic anthropological investigations. XRF is one method of elemental analysis that has been used with increasing frequency, in part due to its ease of use and the fact that little to no sample preparation or destruction are required. XRF analyses have traditionally been performed in a laboratory setting using large benchtop XRF spectrometers. These machines offer a variety of benefits including the ability to control and vary the testing atmosphere. Recently, portable or hand-held XRF units have been developed, with the advantage of being able take the device into the field to perform preliminary or conclusive field tests *in situ* or even for use in a laboratory setting while minimizing the instrument footprint; however, these portable devices may pose potential analytical complications in forensic anthropological cases due to the fact that analyses are all performed in open air, which may affect phosphorus detection since elements of low atomic number are at risk of significant absorption by air. This presentation examines the detection of calcium and phosphorus using two different XRF instruments, a benchtop and a hand-held, and compares results.

Calcium and phosphorus content in osseous and dental material from human and non-human sources were measured by XRF to obtain Ca/P ratios. Samples were cut using a diamond wafering saw to reveal cross sections with flat, smooth surfaces for measurement and included human and non-human (cow, deer, and pig) bones as well as teeth from human, deer, and pig. Shell and coral samples (which are known to have high levels of Ca but little to no P) were also examined. A control sample consisting of calcium hydroxyapatite powder was also analyzed.

Samples were analyzed under three conditions using two XRF instruments. Specimens were analyzed in both air and vacuum atmospheres using a benchtop spectrometer and were analyzed in air using a field-portable XRF. The X-ray tubes of both instruments were operated in unfiltered conditions with an excitation voltage of 20kV. The collection time was maintained consistently at 100 seconds live time. Spectral analysis was performed using instrumental software, and calcium and phosphorus emissions were used to calculate Ca/P ratios using the counts detected in the region of each of the peaks summed over five channels.

Analysis of Variance (ANOVA) shows highly significant differences in Ca/P ratios obtained under the three analysis conditions for individual sample groups as well as for all bones combined. These differences are due in part to overall differences in the detection of both elements, but differences in the detection of phosphorus contribute most significantly. Detection levels for both elements were overall lowest using the hand-held instrument. Detection levels for both elements were greater when using the benchtop in air and greatest when analyses were performed on the benchtop in a vacuum.

Calcium and phosphorus are both measureable under all three analytic conditions included in this study; however, since the calcium and phosphorus signals are attenuated differently by air, comparing measurements made under different atmospheric conditions may be misleading. To use XRF-generated Ca/P ratios to evaluate skeletal versus non-skeletal origin, measurements should be made under consistent analytical conditions. That is, known skeletal and non-skeletal samples should be evaluated to establish the performance of an instrument and specific measurement conditions prior to attempting to evaluate an unknown. When measured under the same conditions, XRF-generated Ca/P ratios are valid criteria for distinguishing differences between material sources.

Forensic Anthropology, Elemental Analysis, XRF

A69 Osteometric Reassociation Through Quantifying Long Bone Size and Shape and Prediction Using Bayesian Regression Via Hamiltonian Markov Chain Monte Carlo (MCMC)

Kyle A. McCormick, MA, 805 Deery Street, Knoxville, TN 37917*

After attending this presentation, attendees will be informed about methods for quantifying long bone morphology and using these data in a predictive framework for resolving small-scale, closed-population commingled assemblages.

This presentation will impact the forensic science community by integrating information on long bone shape into osteometric sorting models that largely rely on size as assessed through standard osteological measurements. Additionally, this presentation introduces a novel predictive framework for osteometric reassociation: Bayesian regression via Hamiltonian MCMC.

Commingled assemblages present a common situation in osteological analysis where discrete sets of remains are not readily apparent, thereby hindering biological profile construction and the identification process. Of the methods available for resolving commingling situations, osteometric sorting is reliable and relatively objective.[1] Current osteometric sorting methodology models long bone relationships by calculating a distance measure from standard osteological measurements, transforming that distance measure into a test statistic, and evaluating the value of that statistic against the appropriate distribution to arrive at a *p*-value.[1] This methodology is a decision-making, error-mitigation approach, where possible matches are eliminated if the accompanying *p*-value exceeds an analyst-defined threshold.[1] Elements are reassociated if all other possibilities are eliminated and the assumption of a closed-population is met (see reference 1 for a more nuanced consideration of this approach and its statistical foundation).

The primary goals of the current study are three-fold: (1) examine the reliability (as assessed through correct classification rates) of a predictive framework for reassociation; (2) study the utility of predictive probabilities and typicality values as metrics for reassociation; and, (3) incorporate information on long bone shape from geometric morphometric landmark data into osteometric reassociation models.

To accomplish these goals, landmark data were collected from the long bones of 208 subjects, males (n=103) and females (n=105), between 19 years and 62 years of age at death, from the William M. Bass Donated Skeletal Collection. Raw landmark data were fit using generalized Procrustes analysis to extract log-centroid size and Procrustes coordinates. Procrustes coordinates were subjected to Partial Least Squares (PLS) analysis to extract relevant components. After the sample was transformed into log-centroid size (size variable) and PLS components (shape variables), ten individuals were randomly removed from the total sample, acting as a small-scale, closed-population commingled assemblage. One element was chosen from the commingled assemblage as the independent variable, with the ten possible matching elements representing the dependent variable. Using the remaining total sample, Bayesian regression via Hamiltonian MCMC was used to estimate a range of possible dependent variable values. These values were smoothed into a probability density function using kernel density estimation and the ten possible matches were evaluated against this distribution to calculate predictive probabilities and typicality values. The element with the highest predictive probability was considered the best match. Femur antimere comparisons illustrate the utility of this approach.

Over the course of 1,000 simulations, matches were correctly classified for 77.6% of the commingled assemblages. When size and shape were analyzed separately, correct classification dropped to 51.1% and 60%, respectively. Despite the high classification rate, predictive probabilities for correctly classified matches were equivocal, with a mean value of 0.1755 and a range of 0.1164-0.2973. These values were similar to predictive probabilities of incorrect classification (mean: 0.1502, range: 0.1159-0.2649), suggesting predictive probabilities alone were a poor means of identifying classification error. Typicality values were minimally helpful in identifying classification error, with mean typicality values for correct and incorrect classifications of 0.8541 and 0.6919, respectively. Additionally, typicality values for both correct and incorrect classifications ranged all possible values.

These results suggest that bones can be reliably reassociated using the predictive framework detailed above. The osteometric reassociation model presented incorporates both shape and size information, providing a more complete representation of long bone form over standard osteological measurements. Additionally, Bayesian parameter modeling results in a distribution of possible values for the independent variable, directly modeling uncertainty in its estimation; however, practical applications of this model are currently limited by a means to detect classification error, as predictive probabilities and typicality values are similar for both correctly and incorrectly classified matches.

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Commingling, Geometric Morphometrics, Bayesian Modeling

A70 A Simple Method for Estimating Subject-to-Camera Distance for Legitimate Craniofacial Superimpositions

Carl N. Stephan, PhD, The University of Queensland, School of Biomedical Sciences, Saint Lucia, Queensland 4072, AUSTRALIA*

After attending this presentation, attendees will be introduced to a simple method for estimating face-to-camera distance from frontal facial photographs using face anatomy alone (palpebral fissure length) when focal length of the lens is known. This presentation will also compare accuracy of these results to those acquired using camera-to-subject distances extracted from the metadata of the corresponding digital image files (another technique that so far has gone uncommented upon in the superimposition literature). Normally, the inclusion of an inanimate object, or smiling expression with a view of teeth, in a facial photograph is thought to be required for matching skull-to-camera distance to face-to-camera distance in craniofacial superimposition. This is not true.

This presentation will impact the forensic science community by providing new simple methods for matching skull-to-camera to face-to-camera distances, as required for perspective distortion matching, when undertaking one-to-one anatomical comparisons in craniofacial superimposition.

Like any forensic science technique, video superimposition should be a scientifically robust procedure, subject to strict performance criteria. One of these criteria must be that the perspective distortions between the two images that are superimposed are the same, so that one-to-one anatomical comparisons can be undertaken. This requires knowledge about what subject-to-camera distance was used to acquire the facial photograph under analysis, but presently this is said to be impossible unless the photographer who took the photograph can be contacted (e.g., Sekharan says, "it is almost an impossible task to determine (subject-to-camera) distance exactly from the photograph").[1]

This study took frontal photographs of four subjects in the "lip shut" posture (one adult male, one adult female, one sub-adult female, and one juvenile male) with a known objective lens length (100mm) at distances between 1m and 10m serially increasing by 1m and used a relatively invariant facial trait (palpebral fissure length) to calculate face-to-camera distance with the formula: $d=f(1+a/b)$, where d=face-to-camera distance (m), f=focal length (mm), a=real-life object size (mm), and b=object size on the image receptor.

Using this method across all ten measurement scenarios for each subject, results indicate a mean percentage error of 7% (range=3.5%-10%) for face-to-camera distance estimation, which falls well within the tolerance levels to obtain <1% difference in facial dimensions at life size due to perspective distortion differences between photography sessions. Palpebral fissure measurement is, thereby, verified as a suitable method for gauging face-to-camera distances. Compared to subject-to-camera distance information extraction from the photographic file's metadata, the palpebralfissure measurement performed superiorly with only 8% absolute error compared to 23% error for the metadata.[2] This unambiguously verifies the palpebral fissure measurement as useful for craniofacial superimposition and as a cross-check of recalled photographic parameters by photographers reporting to have taken the original facial photograph.

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Forensic Anthropology, Video Superimposition, Perspective Distortion

A71 Texture Mapped Average Skulls Created From Standardized Photographs Using the Perception Lab's Psychomorph

Jodi M. Caple, BS, The University of Queensland, School of Biomedical Sciences, Saint Lucia, Queensland 4072, AUSTRALIA; and Carl N. Stephan, PhD, The University of Queensland, School of Biomedical Sciences, Saint Lucia, Queensland 4072, AUSTRALIA*

After attending this presentation, attendees will be aware of highly realistic, sex-specific, mathematically average photographs of skulls for South African Black and White, American Black and White, and Japanese population groups.

This presentation will impact the forensic science community by providing, for the first time, photographic-quality average skulls to objectively illustrate skull morphotypes.

To date, "typical" skulls for each group have been represented either by average linear measurements or Cartesian coordinates, descriptions of morphoscopic trait frequency, or diagrams (drawings or photographs) of single skulls thought to represent the morphotype well. While these methods are important for determining sex and ancestry to aid in identification, they do not allow accurate visualization of what the average skull for each group would look like. Current depictions of each group rely either on diagrams that caricaturize typically observed morphoscopic traits or on a single individual as representative of an entire population. The central tendency, being the most widely used measure for normally distributed data, is a natural choice to more accurately visualize depictions of skulls classified according to particular groups. This was undertaken for this study by: (1) taking standardized photographs of skulls in anterior and left lateral views; (2) outlining the skull shape; (3) calculating the average shape; (4) warping individual photographs to the average shape; and, (5) averaging the color information of the warped photographs to obtain the final average shape and color result.[1] This method has previously been successfully applied to face photographs, producing average faces for individuals (males and females) of self-reported European and Central/Southeast Asian origins.[2]

Standardized photographs were taken of skulls in an anterior view and left lateral view, sourced from the Pretoria Bone Collection, the Hamann-Todd Collection, and the Chiba Bone Collection. Photographs were taken using a full-frame Digital Single-Lens Reflex (DSLR) camera fitted with a 100mm lens, and a camera-to-object distance of 1.2m. Each image was manually delineated by positioning landmarks that were then joined with contour lines to form the outline delineation map. This map was created for each and every skull photograph in this study, and the map was specific to the photographic view. The average x and y coordinate position of each landmark was then used to form the average delineation map, which was used to warp each individual image to the average shape. The average color information for each pixel was then applied for each group to produce the final images.

These average images provide the first quantified basis for depicting skulls grouped by sex and ancestry. They can serve as exemplar images for sex and ancestry, eliminating the need for caricaturized diagrams or isolated single examples drawn out of the sample distribution.

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Anthropology, Photography, Reference

A72 New Forensic Archaeological Recovery Protocols for Fatal Vehicle Fires

Alexandra R. Klales, PhD, Mercyhurst University, Dept of Applied Forensic Sciences, 501 E 38th Street, Erie, PA 16546; Dennis C. Dirkmaat, PhD, Mercyhurst University, Dept of Applied Forensic Sciences, 501 E 38th Street, Erie, PA 16546; and Luis L. Cabo, MS, Mercyhurst University, Dept of Applied Forensic Sciences, 501 E 38th Street, Erie, PA 16546*

After attending this presentation, attendees will be familiar with new forensic archaeological protocols applicable to fatal vehicle fire scenes that result in the efficient and effective recovery of human remains and associated evidence.

This presentation will impact the forensic science community by highlighting the benefits of employing modified forensic archaeological techniques in the documentation and recovery of the fatal vehicle fire scene. Furthermore, the forensic science community can employ the detailed recovery protocols being presented for fatal vehicle fire recoveries.

Fatal fires are one of the most complex scenes to recover, primarily because materials at the scene are heavily modified. In particular, human remains appear homogenous in color with the surrounding burned matrix and they become very fragile due to the taphonomic modifications of fire and subsequent suppression efforts by first responders. Too often, remains are quickly pulled from the fire debris with no documentation of body positioning or of the relationship of the remains to other physical evidence. The complexity of fatal fire scenes and the current recovery protocols for these types of recoveries often result in the human remains and evidence being missed, damaged, or destroyed in their entirety.[1] This in turns hinders autopsy, positive identification, and laboratory and bone trauma analysis of the remains.

Of approximately one million fires per year in the United States, 13.3% are vehicle fires.[2] Fatal vehicle fires are hybrid scenes with combined characteristics of both indoor and outdoor scenes. Much like indoor scenes, the general structure of the burned vehicle is preserved, thereby providing permanent reference points and simplifying mapping; however, the quantity of material, impact of taphonomic agents, and the potential stratigraphic relationships of remains and evidence are all features consistent with outdoor crime scenes. There are other complexities unique to vehicle fires that warrant specific recovery protocols that differ from indoor scenes, outdoor scenes, or fatal structure fires. First, vehicle fires almost always have accelerants present (i.e., gasoline) that impact how the vehicle burns. Second, components of the vehicle, such as the magnesium steering column, create an exploding effect that mirrors the dispersal of materials more commonly encountered with bomb blasts or mass disaster scenes. Last, vehicle fires are more contained and are somewhat easier to process than structure fires due to their smaller size. Often, it is easier to identify the location of the human remains very quickly in vehicle fires.

The new protocols being presented for fatal vehicle fire recoveries are based on and modified from the protocols developed by Dirkmaat et al. for burned structures and are based on field exercises and past case studies.[3] A summary of the newly developed protocols is as follows: (1) overall scene documentation (photographic, written, and spatial team with barcoding system) that continues throughout recovery; (2) pedestrian line search to flag evidence and remains; (3) determination of significance; (4) preparation of the vehicle for recovery (i.e., removal of the roof, trunk, and doors); (5) establishment of a mapping system (i.e., subdivide vehicle into regions using a baseline or grid system); (6) excavation of individual units using trowels, dustpans, and labeled buckets; (7) hand sorting of debris on tarps and screening through ¼" mesh; (8) full exposure of the remains *in situ*; (9) creation of a plan-view map detailing positioning of the remains and associated evidence; (10) mapping of the grid system by the provenience team to later be geo-referenced to the hand-drawn map; (11) wrapping of loose elements and fragile bones with heavy-duty plastic wrap; (12) removal of the remains using body bags and backboards to stabilize the body during transport; (13) collection of loose pieces in labeled containers or bags for re-association in the laboratory; and, (14) excavation beneath the remains following removal.

The implementation of these new protocols using modified forensic archaeological methods results in a nearly 100% recovery of remains and physical evidence in a timely manner, while also limiting disturbance and damage of the remains during the recovery of fatal vehicle fires. Furthermore, detailed mapping and photographs provide precise information on contextual relationships of the remains and evidence at the scene following recovery.

This project was funded in part by the National Institute of Justice, United States Department of Justice.

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Vehicle Fires, Forensic Archaeology, Recovery Protocols

A73 Forensic Examination of Burned Human Skeletal Remains: Shifting the Paradigm

David Gonçalves, PhD, Research Centre for Anthropology and Health, University of Coimbra, Coimbra, PORTUGAL; João Pedro Valente de Oliveira Coelho, MSc, University of Coimbra, Dept of Life Sciences, Calçada Martim de Freitas, Coimbra 3000-456, PORTUGAL; Calil Makhoul, MSc, University of Coimbra, Dept of Life Sciences, Calçada Martim de Freitas, Coimbra 3000-456, PORTUGAL; Inês Santos, MSc, University of Coimbra, Dept of Life Sciences, Calçada Martim de Freitas, Coimbra 3000-456, PORTUGAL; Ana Vassalo, MSc, University of Coimbra, Dept of Life Sciences, Calçada Martim de Freitas, Coimbra 3000-456, PORTUGAL; Maria Teresa Ferreira, PhD, Forensic Sciences Centre, University of Coimbra, Coimbra, PORTUGAL; Luis A.E. Batista de Carvalho, PhD, University of Coimbra, Dept of Chemistry, Molecular Physical-Chemistry R&D Unit, Coimbra 3004-535, PORTUGAL; and Eugenia Cunha, PhD, Universidade de Coimbra, Dept of Life Sciences, Laboratory of Forensic Anth, Calçada Martim de Freitas, Coimbra 3000-456, PORTUGAL*

After attending this presentation, attendees will better understand Heat-Induced Changes (HIC). Additionally, current methods for assessing the biological profile, when applied to burned skeletal remains, will be improved.

This presentation will impact the forensic science community by proposing new approaches to the analysis of burned skeletal remains and by providing knowledge obtained from the research carried out in the framework of the ResearcH PrOject of the 21st-Century Skeletal Collection (CEI/XXI) Burned SkeleTons (HOT).

The forensic analysis of burned skeletal remains is often complicated by HIC because they interfere with the application of standard methods. Probably the most obvious difficulties are related to fragmentation and to the inability in assessing the extent of HIC — namely concerning dimensions and warping — affecting a particular bone or tooth. Despite the increase in the amount of research, a reliable method to estimate this extent is still escaping us. At the University of Coimbra in Portugal, skeletons from the CEI/XXI are being experimentally burned under controlled conditions.[1] The main objectives of this project are: (1) to achieve a better understanding of HIC; (2) to test the reliability of current methods for assessing the biological profile, when applied to burned skeletal remains; and, (3) to develop new analytical methods more specific to burned skeletal remains according to the extent of burning.

Unclaimed skeletons from a public cemetery donated to the University of Coimbra are being compiled, allowing for invasive procedures such as burning. Only the right antimeres of each skeleton are subject to controlled burning (up to 1,050°C) while unpaired bones such as the cranium are not being burned. The unburned bones serve as a basis for comparison. Analyses of the skeletons are performed before and after burning to document color, weight, and morphological and dimensional changes. Comprehensive research on burned skeletal remains is already underway, although the current sample size is still small (n=20).

The potential of cementochronology for age estimation on calcined teeth has been investigated. Although the estimated age through Tooth Cementum Annulations (TCA) presented poor agreement with chronological age (mean error=24.2 years), partly due to the poor visibility of the lines, a new cementochronological approach based on the estimation of the amount of TCAs present in each tooth provided better results (mean error=11.4 years). In addition, an attempt to determine the effect of bone collagen on the occurrence of heat-induced warping was conducted. Although a slight significant effect (p=.040) was indeed found, multivariate statistics identified other more significant factors in the model: maximum temperature (p<.001); duration of combustion (p<.001); sex (p<.001); and age at death (p=.010). The results demonstrated that warping can occur on bones with both low and highly preserved collagen contents, suggesting that the role of the burning dynamics is particularly important. As a result, warping appears to be an unreliable indicator of the pre-burning condition of skeletal remains (fleshed vs dry). In another research project, the focus is on the potential of geometrics morphometrics to assess the pre-burning shape and size of burned bones. A multivariate approach is being adopted to determine if these parameters — usually impossible to assess during the examination of burned remains — can be reliably obtained through virtual retro-deformation. Initial results show some promise, although additional research is needed.

Several incidents involving fire (e.g., airplane crashes, terrorist attacks, bush fires) can result in victims whose remains are skeletonized and burned. Experimentation with human remains is critical since other species may not serve as reliable proxies. A good documentation of HIC and the validation of analytical methods are also fundamental. Controlled laboratory burnings do not always replicate the usual on-and-off burning exposure occurring in some forensic scenes; also, dry bones may react differently than fleshed or green bones.[2] These are some of the shortcomings of this new collection, but it still allows important insights about HIC, and its contribution for the improvement of more adequate bioanthropological methods for the analysis of burned bones and teeth is undisputable.

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Burned, Skeleton, Forensic

A74 Remote Sensing of Human Burials

Katie Corcoran, BS, 250 S Stadium Hall, Knoxville, TN 37996; Amy Z. Mundorff, PhD, University of Tennessee, Dept of Anthropology, 250 S Stadium Hall, Knoxville, TN 37996; Devin White, PhD, Oak Ridge National Laboratory, 1 Bethel Valley Road, Oak Ridge, TN 37830; and Whitney Emch, PhD, National Geospatial-Intelligence Agency, 7500 Geoint Drive, Springfield, VA 22150*

After attending this presentation, attendees will understand some principles of remote sensing, some characteristics of human burial disturbance that are observable in remotely sensed data, and an example of some analytical approaches to separating disturbance signatures from non-disturbance signatures using an unmarked burial dataset collected at the University of Tennessee Anthropology Research Facility (ARF).

This presentation will impact the forensic science community by providing documented evidence of topographic and spectral signatures for use in narrowing areas-of-interest thought to contain buried human remains, as well as other types of ground disturbances. Further, this research is unique in that it separates signatures of truly clandestine disturbance targets by comparing like-materials (e.g., affected live vegetation to unaffected live vegetation).

In addition to an empty control grave, ten donated human bodies were buried in three differently sized graves at the ARF in February 2013, for a total of four disturbance targets. In 2013 and 2014, multiple terrestrial Light Detection and Ranging (LiDAR) and spectral datasets were collected using a tripod-mounted laser scanner and a portable spectroradiometer (350nm-2,500nm), respectively. These datasets were subjected to rigorous statistically based data reduction methods to maximize their separability. LiDAR point clouds were filtered using pre-defined local elevation thresholds to remove non-ground points, and reflectance spectra were filtered to remove wavelength bands not significantly contributing to a binary presence/absence disturbance classification in training data.

Filtered LiDAR data reveal distinctive burial footprints, expressed as subtle depressions that are especially apparent in elevation change images and range from -10cm–0cm. Elevation loss is most pronounced directly over buried human bodies due to the redistribution of mass from decomposition, in addition to normal soil settling. Unsurprisingly, these depressions fill in with debris over time, resulting in diminished visibility; however, the two largest burials were still clearly visible in LiDAR data collected at 22 months post-burial.

Statistical analysis of spectra reveals separation in the visible and infrared regions of the spectrum. A Discriminant Function Analysis (DFA) results in the correct classification of 60.0% of disturbed vegetation (n=70) and 54.8% of non-disturbed vegetation (n=84) in the spring season and 74.1% of disturbed vegetation (n=54) and 60.8% of non-disturbed vegetation (n=79) in the autumn season using cross-validation. Binary Logistic Regression (BLR) results in the correct classification of 54.3% of disturbed vegetation (n=35) and 76.2% of non-disturbed vegetation (n=42) in the spring season and 55.6% of disturbed vegetation (n=27) and 74.3% of non-disturbed vegetation (n=39) in the autumn season using a separate validation sample. It is likely that a hybrid statistical model will successfully exploit the high rate of correct DFA disturbance classification and the high rate of correct BLR non-disturbance classification to achieve optimal results. Experimentation with machine-learning approaches suggests they may be useful for reinforcing statistically based predictive models under certain seasonal and technological conditions.

This presentation will demonstrate the importance of using multiple datasets for isolating small or subtle targets when it makes sense to do so, as it does in clandestine human burial scenarios. This presentation will highlight important considerations for these findings, including how ground-based data can be used on its own or in combination with other intelligence and data to inform remote aerial and orbital data collections. Additionally, this presentation will cover some practical ways investigators might use these findings on the ground to facilitate rapid decision making.

Forensic Archaeology, Remote Sensing, Victim Recovery

A75 The Use of Near-Infrared Remote Sensing in the Detection of Clandestine Human Remains

Marilyn Isaacks, BA, Texas State University, 15931 Watering Point Drive, San Antonio, TX 78247; and Daniel J. Wescott, PhD, Texas State University, Dept of Anthropology, 601 University Drive, San Marcos, TX 78666-4684*

After attending this presentation, attendees will better understand the potential uses of unmanned aerial drones in the detection of clandestine human remains during search missions.

This presentation will impact the forensic science community by introducing a new use for existing drone technology that will allow forensic investigators to quickly, inexpensively, and safely search for clandestine human remains.

Most commonly, searches for clandestine remains have utilized time-consuming methods such as line searches that require the support of many individuals to scour a typically large area. While these methods do yield results, they take time to execute and, in certain places, may actually prove dangerous for the participants.[1] Many additional methods have been tested and utilized in the recovery of human remains, including the use of metal detectors, aerial photography, and ground-penetrating radar, which can be time consuming and expensive.[2] Only in recent years has the use of Near-Infrared (NIR) imagery been experimented with as a means of uncovering clandestine graves and surface remains.[1-3]

As human remains decompose, a large amount of organic matter enters into the surrounding soil, forming a Cadaver Decomposition Island (CDI).[4] Because soils that are organically rich have a different reflectance signature than nearby unaffected soils when viewed with NIR imaging, it is likely that by using NIR photography and drone technology, clandestine remains may be recovered more quickly and more efficiently than has previously been possible.[3] Because NIR photographs can be obtained using small, remotely controlled aircraft or aerial drones, large areas can be surveyed for clandestine remains remotely and rapidly, thereby minimizing the need to involve a substantial group of people in the search. In so doing, potentially dangerous locations can be searched without great risk, disturbances to forensically significant sites will be minimized, and the area that personnel must search will be reduced and more precisely understood.

The present study explores the utility and longevity of NIR cameras mounted to Unmanned Aerial Systems (UAS) in the detection of clandestine human remains. Aerial NIR photographs and soil samples were compiled from 104 identifiable CDIs (i.e., the fertile soil area below and surrounding a decomposing cadaver) at the Forensic Anthropology Research Facility (FARF) at Texas State University in San Marcos, TX. Four surface soil samples were taken from each CDI on the day of the first drone flyover, two from the center, and two from the edge. Half of the soil samples collected from the center of each CDI were sent for analysis of organic materials (specifically organic carbon and nitrogen) at Texas A&M, Department of Soil and Crop Science, while the other half were burned in a muffle furnace at Texas State University to estimate the amount of total carbon within each sample based on the difference between initial soil weight and the ash weight. Each area of FARF that contains or once contained human remains was photographed using a camera with NIR capabilities mounted on UAS. Unused areas of FARF were also examined to determine whether natural disturbances create signatures similar to that of human decomposition.

Results of an unpaired *t*-test show a significant difference ($p<0.001$) between the NIR spectra signature of true placements (i.e., CDIs) versus that of control sites. Baseline trends indicate that CDIs that are more than approximately two years old cease to be visible in the NIR spectra, while CDIs that are younger than two years, but older than one week, are easily distinguishable from the surrounding soil. While no single chemical factor in the present study has a significant effect on the strength of the NIR signature of a given CDI, a multiple linear regression of organic carbon, total nitrogen, and total carbon presents a strong correlation coefficient ($R=0.670$) between the variate and the strength of the NIR spectra signature. Analysis of Variance (ANOVA) results further indicate that this model does significantly predict the strength of the signature (F (3, 58)=14.647, $p<0.001$).

In conclusion, this study demonstrates the utility and efficiency of unmanned aerial drones mounted with NIR-capable cameras in the remote detection of clandestine human remains. The combination of organic materials such as nitrogen and carbon purged from the body during decomposition creates a unique signature that is visible in the NIR spectrum up to two years after its creation and can be used as a tool on search missions for clandestine human remains.

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Clandestine Remains, Aerial Drones, Remote Sensing

A76 The Interpretation of Human Pediatric Cranial Fracture Patterns Using Experimentally Generated Porcine Ground-Truth Data

Jennifer M. Vollner, MS, 354 Baker Hall, East Lansing, MI 48824; Caitlin C.M. Vogelsberg, MS, Michigan State University, Dept of Anthropology, 354 Baker Hall, East Lansing, MI 48824; Patrick E. Vaughan, BS, Michigan State University, Orthopaedic Biomechanics Laboratories, E Fee Hall, Rm 407, East Lansing, MI 48824; Todd W. Fenton, PhD, Michigan State University, Dept of Anthropology, 354 Baker Hall, East Lansing, MI 48824; Steven C. Clark, PhD, Occupational Research and Assessment, 124 Elm Street, Big Rapids, MI 49307; and Roger C. Haut, PhD, Michigan State University, Orthopaedic Biomechanics, A407 E Fee Hall, East Lansing, MI 48824*

After attending this presentation, attendees will be aware of: (1) the existence of the Pediatric Cranial Fracture Pattern Registry (PCFPR); (2) the applicability of experimental porcine cranial fracture-pattern data to human pediatric forensic cases; and, (3) the medicolegal implications of these data for cranial fracture-pattern interpretation.

The presentation will impact the forensic science community by demonstrating the value of ground-truth porcine experimental data in the interpretation of pediatric deaths involving blunt force cranial trauma when the injury history is unknown or of questionable reliability.

Pediatric deaths involving cranial fractures are challenging cases and increasingly call for a multi-disciplinary investigation to interpret injury mechanism and aid forensic pathologists in the determination of the manner of death. In response to these challenges, the PCFPR was recently established at Michigan State University and contains 206 de-identified pediatric death cases involving blunt force cranial trauma submitted by 15 partnering medical examiner offices. This resource was established to provide investigators with an assessment tool that links fracture patterns with provided injury scenarios.

Because limited human experimental ground-truth data exists linking cranial fractures with known scenarios, this study's laboratories have developed a porcine model and performed a series of studies to investigate the effects of surface shape and energy level on cranial fracture initiation and patterns.[1,2] With this foundational work in place, it is now necessary to begin relating the experimental findings to human forensic cases. This study applied the findings from previous research to interpret cases in the PCFPR. It was hypothesized that it would be possible to distinguish cranial fracture patterns produced by focal blunt impacts from fracture patterns generated by impacts onto a flat surface based on this previous research.

A total of 86 homicide cases with fracture pattern diagrams and forensic pathology observations were examined to determine whether the injuries were consistent with flat or shaped impact surfaces. Methods employed in this study were developed from the results of the aforementioned research projects in which infant porcine specimens were subjected to impacts at the center of the right parietal from various shaped implements including: flat surfaces, a 90^{o} edged surface, a 2-inch diameter sphere, a five-eighths-inch diameter sphere, and a one-quarter-inch diameter flat-ended cylinder. These studies demonstrated that fracture patterns are dependent on impact surface shape. Specifically, flat surfaces result in peripherally initiated fractures whereas more focal contact surface shapes resulted in more fractures initiating at the point of impact, curvilinear fractures, and/or areas of depression. These same fracture patterns were investigated in the PCFPR homicides.

The results of this study include the classification of these 86 cases into the following categories: impacts to flat surfaces, non-flat/shaped surfaces, and those that could not be presently classified. Twenty-seven cases (31%) had peripheral fractures, which were most consistent with flat surface impacts, while 24 cases (28%) expressed injuries characteristic of shaped impact surfaces, either in isolation or in conjunction with one another. This is a conservative estimate as several cases exhibited confounding cranial fractures, making them difficult to classify. This left 35 cases (41%) unclassified. Of the cases classified as non-flat, 13 (15%) had fracture patterns consistent with point-of-impact fracture initiation, 8 (9%) presented curvilinear fracture patterns, and 14 (16%) expressed areas of depression. It is noteworthy that several of the non-flat impact cases had injury histories that conflicted with the fracture patterns (e.g., a claimed fall from a couch with non-flat fracture characteristics).

The understanding of fracture characteristics and the mechanisms that cause them have significant implications for forensic investigators. The presence of areas of depression, curvilinear, and/or point-of-impact fracture initiation indicated a high likelihood of focal implement impacts, not a flat surface. This information can be pivotal when attempting to determine the mechanism of pediatric cranial blunt force injuries, especially in cases of suspected neglect or abuse.

This project was supported by a grant from the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect the views of the Department of Justice.

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Cranial Fractures, Blunt Force Trauma, Fracture Biomechanics

A77 Pediatric Antemortem Healing Standards Based on Microscopic Analysis of Fractures in Known Forensic Child Abuse Cases

Donna C. Boyd, PhD, Radford University, Forensic Science Institute, PO Box 6939, Radford, VA 24142; Sharon Roller, 56 Harrison Avenue, Waldwick, NJ 07463; and Cliff Boyd, PhD, Radford University, Dept of Anthropological Science, Radford, VA 24142*

The goal of this presentation is to examine the theoretical and anatomical foundations for pediatric antemortem fracture healing and to present microscopically derived healing standards for use in forensic antemortem trauma cases.

This presentation will impact the forensic science community by providing forensic anthropologists and pathologists with more realistic and useable standards for classification and interpretation of forensic pediatric antemortem fractures. This will aid in determination of time-since-injury for these fractures and provide evidence relating to determination of accidental vs. non-accidental etiology for peri-mortem fractures in pediatric death investigations.

This presentation reviews the theoretical and anatomical basis for antemortem healing, particularly in regard to subadults. The process of bone healing from trauma is a complex and dynamic one which does not lend itself well to compilation of finite stages. Numerous phases/stages for antemortem fracture healing have been presented in the medical and forensic literature and are commonly used by forensic anthropologists and pathologists in determination of time-since-injury in pediatric death investigations.[1,2] These assessments heavily influence determinations of accidental vs. non-accidental origin of peri-mortem pediatric fractures. A comparison of these healing phases reveals inconsistencies in their terminology and definition, in part due to reliance on clinical (non-forensic) samples imaged through radiology. Prosser doubts the relevance of these radiological standards to forensic cases because they are based on immobilized fractures in otherwise healthy children examined in a clinical setting.[3] McCormick and Love have called for revised guidelines for antemortem fracture interpretation based on macroscopic forensic samples.[4]

More accurate observation of the antemortem healing process is accomplished through microscopy. In this study, evidence for this healing process is assessed microscopically in a sample of 679 images taken from a digital light microscope, the Keyence VHX-1000 at 5x-200x, representing 48 fractures from five known cases of pediatric death from child abuse. Forty-one of these fractures are present on ribs; the remainder affect long bones and the clavicle. These cases represent the range of the healing process (early to late); in two cases, fairly precise healing times are known or inferred.

These microscopic images are used to illustrate the healing process and develop microscopically based guidelines for bone healing. Key microscopic signatures observed include evidence for acute inflammation, fracture margin rounding, early beginnings of subperiosteal new bone formation, progressive organization of immature fibrous bone leading to bridging and eventual replacement of woven with lamellar bone, resorption of necrotic bone tissue, loss of fracture margins, and advanced signs of remodeling. A series of images showing this bone healing progression is presented.

Based on this analysis, it is proposed that antemortem healing is a continuous process which is not conducive to rigid interpretation using a finite staging system. Stages, as defined, should be used as an interpretive model representing this continuous process.

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Pediatric, Antemortem Healing, Microscopic

A78 Understanding the Role of Contact Area in Adult Cranial Fracture Variation

Mariyam I. Isa, BS, Michigan State University, Dept of Anthropology, 354 Baker Hall, East Lansing, MI 48824; Todd W. Fenton, PhD, Michigan State University, Dept of Anthropology, 354 Baker Hall, East Lansing, MI 48824; Patrick E. Vaughan, BS, Michigan State University, Orthopaedic Biomechanics Laboratories, E Fee Hall, Rm 407, East Lansing, MI 48824; and Roger C. Haut, PhD, Michigan State University, Orthopaedic Biomechanics, A407 E Fee Hall, East Lansing, MI 48824*

After attending this presentation, attendees will better understand the relationship between skull-implement contact area, location of fracture initiation, and fracture characteristics in controlled blunt impact experiments performed on adult human heads.

This presentation will impact the forensic science community by helping to clarify the role of implement shape in blunt force impacts, which will inform investigators' interpretation of adult cranial trauma.

The purpose of this presentation is to present new data on shaped impact experiments with adult human heads and to examine contact area between the cranium and an impacting surface and its effect on fracture initiation and fracture characteristics.

Seven unembalmed adult male heads were hit in a series of controlled blunt impact experiments, according to a protocol discussed previously.[1] Impacts were delivered to the center of the right parietal using various shaped implements. These included two flat (3" diameter and 1" square) and two curved (2" diameter hemispherical and 1" diameter spherical) aluminum impactors. High-speed photography captured fracture initiation and propagation in these experiments.

Contact area data was recorded for each impact experiment. Pressurestat contact paper was laid onto the center of the parietal and an impact was delivered at a pre-failure level. The resulting contact area impression was measured using digital calipers. This procedure was repeated ten times per specimen-implement pair to obtain average contact area. Additionally, one specimen was Computed Tomography (CT) scanned and computational Finite Element Analysis (FEA) was performed using Abaqus/CAE standard software to model the effect of contact area on maximum principal stresses.

The results showed that implement shape influences contact area. Significant differences in average contact area were recorded between the 1" spherical (2.88±1.02mm^2), 2" hemispherical (9.72±2.42mm^2), and 3" flat (39.38±13.89mm^2) implements. In impacts with two different specimens, the 1" flat implement produced significantly different contact areas (12.78±8.51mm^2 vs. 58.29±9.90mm^2). This indicated the importance of cranial curvature; an impact with the same implement results in a smaller contact area on a more curved cranial surface than a less curved surface.

Contact pressure generated under the Point Of Impact (POI) varied directly with contact area and corresponded with location of fracture initiation and fracture type produced. The 1" spherical implement generated the smallest contact area and highest pressure (2068±633MPa). This was the only impact to produce depressed fracture. Contact pressure in the 2" hemispherical impact was significantly lower (796.6±221MPa). High-speed photography showed that this impact resulted in a POI-initiated linear fracture, followed by a curvilinear fracture near the POI. Pressure in the 3" flat impact was significantly lower than .in either curved impact (232.3±66.4MPa). In this impact, a linear fracture initiated peripherally rather than at the POI.

When contact area was large, an impact with the 1" flat implement generated pressure statistically similar to the 3" flat impact (207.9±34.4MPa) and produced a similar pattern of fracture (peripheral-linear). When contact area was small, an impact with the 1" flat implement generated pressure statistically similar to the 2" hemispherical impact (558.3±308.9MPa). In this case, fracture also initiated at the POI.

Computational modeling helped explain the fracture data. FEA models showed that a large contact surface produced low stresses at the POI, but high tensile stresses at the cranial sutures; this resulted in the initiation of linear fracture peripheral to the POI. At the same energy, a small contact area produced higher stresses and failure at the POI, potentially with depression.

The results of this study suggest contact area, more precisely than implement shape, may explain variation in fracture patterns observed in adult cranial impacts. At similar energy levels, contact area determined the pressure generated under the POI. Contact pressure, in turn, influenced fracture initiation (POI vs. peripheral) and fracture type (depressed vs. linear). Contact area decreased with decreased implement size, but also with increased cranial curvature. These experimental results may help explain differences in cranial fracture patterns observed between individuals or in impacts to different areas of the skull.

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Blunt Force Trauma, Cranial Fracture, Trauma Biomechanics

A79 Dismemberment Injuries: The Contribution of Bone and Soft Tissue Histology

Tania Delabarde, PhD, Institut de Médecine légale, 11 rue Humann, STRASBOURG 67085, FRANCE; Catherine Cannet, 11 Rue Humann, Strasbourg 67065, FRANCE; Annie Geraut, MD, 11 Rue Humann, Strasbourg 67000, FRANCE; Marc Taccoen, MD, Institut Médico-Légal, 2 Place Mazas, Paris 75012, FRANCE; Bertrand P. Ludes, MD, PhD, Institut Medico- Legal, 2 Place Mazas, Paris 75012, FRANCE; and Jean-Sébastien Raul, 11 Rue Humann, Strasbourg, AE 67085, FRANCE*

After attending this presentation, attendees will better understand the potential of histological analyses on bone and soft tissues from dismembered victims.

This presentation will impact the forensic science community by providing three cases of documented dismemberment injuries that exemplify the contribution of histology to direct the investigation and bring evidence to the courtroom.

Dismemberment, the intentional separation of body segments, is known to be one of the major postmortem activities performed by humans on the remains of others. The victim's body is the foremost source of evidence and attempts to dispose of remains may inhibit identification and destroy links with the perpetrator, the crime scene, and the events leading up to death. Three forensic cases of dismembered victims are presented to discuss relevant autopsy and anthropological and histological findings.

Case Study 1: Dismembered body segments of a young adult male placed in three plastic bags were recovered in the street by a doorman next to a garbage area. During the autopsy, a great number of stab wounds were found and cause of death was determined as multiple stab wounds to the chest (cardiac and pulmonary laceration). The victim also sustained saw marks associated with complete separation at the level of the cervical spine, upper limbs, and lower limbs. Bone sections and cutaneous margins were removed for cut marks analysis and histology. Dismemberment was suspected to be postmortem as no evidence of hemorrhagic infiltration was observed during autopsy and was later confirmed by histopathology results. Numerous false starts and kerfs exhibited characteristics consistent with a power saw. Histomorphometric analyses of bone and soft tissue revealed the presence of exogenous particles (silicon carbide) that belong to a specific power saw blade. This information was crucial for the determination of the tool type used by the perpetrator.

Case Study 2: The remains of a saponified dismembered body from a young female were exhumed from a house basement. Only the lower limbs sustained dismemberment injuries with the complete separation of legs and feet. Bone segments were retained for both anthropological examination of cut marks and histological analysis. Kerf walls exhibited blue particles that were observed macroscopically and microscopically as potential paint residue. Anthropological and histological findings were relevant to identify class characteristics of the offending instrument: a hand saw with a blue-painted blade was finally recovered by police officers in the house of the perpetrator, who confessed to the murder and dismemberment of the victim.

Case Study 3: The saponified body of an adult male was exhumed from a shallow grave. The head was completely severed from the trunk at the level of the second and third cervical vertebra. Autopsy findings were consistent with sharp force injuries at the level of the neck with the use of a blade instrument. Bone and soft tissue were retained for examination of cut marks and histological analysis. Bone examination confirmed the presence of incisions on both vertebrae consistent with sharp force trauma. The presence of exogenous particles was determined on bone and soft tissue samples; their physical characterization was different from geological features from the grave location but consistent with the place of events described by perpetrators.

These three case studies illustrate that bone histology offers great potential for augmenting the investigation and is not limited to age estimation or bone remodeling. Soft tissue and bone samples from traumatic injuries should be microscopically examined as histomorphometric findings are complementary with forensic examination data and could sometimes provide key elements for the investigation.

Histology, Dismemberment, Forensic Anthropology

A80 Evaluating Timing of Injury in Central Florida: Examining the Transition of Fracture Characteristics From Wet to Dry in Long Bones

Ashley Green, MA, 1219 Dowden Street, Charleston, SC 29407; and John J. Schultz, PhD*, University of Central Florida, Dept of Anthropology, 4000 Central Florida Boulevard, HPH 309, Orlando, FL 32816*

After attending this presentation, attendees will better understand the transition of intrinsic properties of bone from wet to dry. This presentation will focus on the timing of injury in the postmortem period in order to fill a gap in the literature regarding the time frame in which bone transitions from wet properties to dry properties in the Central Florida environment by examining fracture characteristics.

This presentation will impact the forensic science community by discussing the timing of the transition of intrinsic properties of bone from wet to dry. Fracture characteristics such as fracture angle, fracture surface, and fracture outline will be discussed in terms of wet and dry characteristics. This will aid the forensic community in differentiating between peri-mortem and postmortem trauma in the elastic peri-mortem period.

Differentiating between peri-mortem and postmortem fractures can be difficult when bone retains fresh characteristics in the Postmortem Interval (PMI). As a result, it is important to conduct research that investigates the timing of the injury in the postmortem period by observing fracture characteristics created at known intervals.[1-4] Investigation into the timing of injury was undertaken over a 14-week time period in Central Florida. By fracturing bones using a custom impact device, specific morphological characteristics typically used in trauma analysis were able to be analyzed: fracture angle, fracture surface, and fracture outline.[1-4] Long bones of pigs (*Sus scrofa*) (N=140) were placed in two outdoor microenvironments: full sun (Group A) and full shade (Group B). Five bones were collected from each microenvironment weekly and subsequently fractured. Additionally, a control group of five fresh bones was fractured immediately to simulate peri-mortem trauma.

Analysis of fracture characteristics was conducted using a standardized protocol modified from previous studies.[1-4] Statistical analyses were performed to investigate the relationships between the variables. The statistically significant results of the Chi-square analysis for the entire data set comparing fracture angle and fracture outline (p=0.000), fracture angle and fracture surface (p=0.003), and fracture outline and fracture surface (p=0.000) indicate that the variables are likely dependent upon one another; however, when microenvironment was considered, the results indicate that fracture angle and fracture outline are likely independent of one another for Group B (p=0.080). Analysis of Variance (ANOVA) testing was conducted for the entire data set, as well as considering microenvironment, using time (the PMI) as a dependent variable. The results denote statistically significant relationships between fracture angle and PMI (p=0.001), fracture surface and PMI (p=0.000), and fracture outline and PMI (p=0.006); however, when environment was considered, the results denote no significant relationship between fracture outline and PMI for Group A (p=0.154). The results of this study indicate a discernable shift in the timing of occurrences of dry characteristics as PMI increases, with a transitional period identified around weeks 5 to 9. Wet characteristics were observed into the 13th week; however, dry characteristics were seen within two weeks postmortem Additionally, statistical analyses indicate that the environment in which bones are deposited has a significant effect on fracture surface and outline as PMI increases.

These results suggest that it is possible to distinguish wet from dry fracture characteristics in Central Florida earlier than previously reported.[1-4] Fracture surface and fracture outline were the most useful characteristics for evaluating the transition from wet to dry. Group B exhibited dry characteristics earlier than Group A, indicating environmental factors are regionally specific and specific to microenvironments. The use of taphonomic models that are regionally specific and standardized protocols for scoring fracture characteristics provides increased accuracy in estimating timing of injury.

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Forensics, Fracture, Trauma

A81 A Test of the Transition Analysis Method for Estimating Adult Age-at-Death

Jessica L. Campbell, MS, 2507 15th Street, Troy, NY 12180; and Stephen P. Nawrocki, PhD, University of Indianapolis, Dept of Biology, 1400 E Hanna Avenue, Indianapolis, IN 46227-3697*

After attending this presentation, attendees will better understand the effectiveness of Transition Analysis (TA) and ADBOU software compared to other well-known age estimation methods for skeletonized remains.

This presentation will impact the forensic science community by providing a rigorous validation test of TA and by identifying the limitations and advantages of various age estimation methods.

Several problems inherent to most age estimation methods include the tendency to overestimate the ages of younger individuals and to underestimate the ages of older individuals, to lump the elderly into an umbrella category of ">50 years," and "age mimicry," or the tendency of a method to produce age estimates that mirror the age distribution of the collection on which the method was developed. Milner's and Boldsen's TA was developed to address and resolve these problems.[1] The method uses Bayesian statistics in tandem with a modified "component" scoring system to produce an age estimate that reflects a decedent's age at transition. The method's software interface, ADBOU, draws from one of two known prior population distributions (forensic and historic hazards) that are preloaded and unalterable. TA scores a number of different components for three skeletal indicators: the Pubic Symphysis (PS), the Auricular Surface (AS), and the Cranial Sutures (CS). TA can therefore be used with incomplete or fragmented remains.

The present study's primary goal is to test the accuracy of TA and ADBOU against traditional "phase" methods commonly used for the same three skeletal indicators: Suchey-Brooks' (PS), Osborne et al. (AS), and Nawrocki (CS). The hypothesis that TA is more effective in older age ranges than traditional methods was evaluated by calculating inaccuracy and bias by decade; Spearman's rho evaluated whether component scores are correlated with actual age better than phase scores; and the effects of continent of origin and ancestry on prediction error were evaluated with Analysis of Covariance (ANCOVA).

This study sample consisted of 147 modern adult males with documented ages at death, drawn from the Pretoria Bone Collection in South Africa (n=72) and the Bass Donated Collection in Tennessee (n=75). Scoring was conducted blind without reference to the actual age of the decedent. Each skeleton was scored using the three traditional phase methods as well as with the three TA component methods. Target age estimates and 95% prediction intervals were obtained for each male using the traditional method's published tables and ADBOU's algorithms for the TA data. Furthermore, summary age estimates were calculated by averaging the results of the three traditional methods, to compare with the corrected age calculated by ADBOU.

Mean prediction error (inaccuracy) is always significantly lower for the three traditional methods than for the three TA component methods, and averaging the three traditional methods produces the lowest mean error in the study (12.5 years, compared to 17.1 years for TA). While average bias was always significantly lower for the three TA component methods, the summary age provided by ADBOU suffers from considerable bias, indicating there may be a problem with the software's algorithm. Evaluation of the older age categories indicates that TA does not provide any clear advantage over traditional methods, although TA does seem to produce slightly more accurate estimates for younger individuals. Spearman's rho shows that component scores, taken individually or summed, are not more highly correlated with age than traditional phase scores. ANCOVA results indicate that residuals from the traditional methods were not influenced by ancestry or continent of origin, while the TA residuals were. These results indicate that: (1) while TA does display lower prediction bias, it does not perform as well as traditional methods with respect to inaccuracy, and therefore, in forensic settings, the traditional methods are preferred; (2) age estimation is more accurate when multiple indicators are averaged; and, (3) despite their lower accuracy, the cranial sutures seem to stabilize the other indicators, offsetting the tendency for the pubic symphysis and auricular surface to underestimate age.

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Age Estimation, Transition Analysis, Biological Profile

A82 Examining the Accuracy of Age Estimates From New Histological Sampling Strategies at the Femoral Midshaft

Timothy P. Gocha, PhD, The Ohio State University, 1645 Neil Avenue, 279 Hamilton Hall, Columbus, OH 43210; Sam D. Stout, PhD, Ohio State University, Dept of Anthropology, 4034 Smith Laboratory, Columbus, OH 43210-1106; and Amanda M. Agnew, PhD, The Ohio State University, 279 Hamilton Hall, 1645 Neil Avenue, Columbus, OH 43210*

After attending this presentation, attendees will understand the spatial variation present in the distribution of intracortical remodeling events throughout the entirety of the femoral midshaft and the importance of selecting Regions Of Interest (ROI) for developing new histological aging methods.

This presentation will impact the forensic science community by introducing new sampling strategies for the quantification of histological remodeling that can be used to estimate age and further demonstrating these age estimates to be highly accurate throughout the adult life span.

As a complement to macroscopic aging methods, or when necessary macroscopic elements are damaged/absent, age can be estimated through histological examination of remodeling events in cortical bone. During the past half century, the femoral midshaft has been the most commonly employed site for histological studies; however, a consensus is still lacking on where to best quantify remodeling, as different methods employ various ROIs that differ in size, number, and location. To address this knowledge gap, this study employed Geographic Information Systems (GIS) software to digitally map all remodeling events (intact and fragmentary osteons and resorptive bays) across the entirety of the femoral midshaft. Patterns in the spatial distribution of remodeling were then examined to identify which region(s) of the femoral cortex produce the most accurate age estimates.

Thirty complete cross-sections from modern cadaveric femora were used, 15 of each sex, ranging from 21 year to 97 years of age (mean=58.9; Standard Deviation (SD)=22.1 years), with both sexes having similar age distributions. Each sample was photographed under polarized light and seamless cross-sectional images were imported into arcGIS® v10.1. Polygon features were created to overlay cortical areas and all remodeling events (n=230,870) were identified and digitally annotated with point features. A total of ten different sampling strategies were employed, each subdividing the entire cortex in a different manner. Osteon Population Density (OPD) was calculated by summing all remodeling events within an ROI and dividing by its area.

Statistical analyses were performed in the Statistical Package for the Social Sciences (SPSS) 21. OPD values were normally distributed for each ROI, and Multivariate Analysis of Covariance (MANCOVA) analyses revealed that OPD was not significantly different between sexes for any ROI, allowing the combination of male and female data for further analyses. Paired t-tests revealed OPD calculations were not statistically different between observers. Stepwise linear regression was used to determine which ROIs from each sampling strategy were most useful in estimating age. To further evaluate the performance of the resulting predictive models, jackknife age estimates were generated by removing an individual from the sample, recalculating the regression model, and then estimating the age of the individual not included in the model; this was done iteratively for all individuals. The accuracy of these estimates was analyzed through measures of bias and inaccuracy.

Results indicate the two most promising sampling strategies are dividing the femoral cortex into Anterior, Posterior, Medial, and Lateral (APML) quadrants separated into periosteal, middle, and endosteal thirds, and also APML octants separated into thirds. Stepwise regression selected four ROIs for each method, primarily in the lateral and anterolateral regions of the cortex, and spread between all depths of the cortex. The resulting model for the APML quadrants by thirds explains more than 90% of the variation in age (adj. R^2=0.907, p=0.000) with a standard error of 6.73 years, while the APML octants by thirds explained more than 93% of the variation in age (adj. R^2=0.931, p=0.000) with a standard error of 5.82 years. Jackknife age estimates from both models were very promising, with average differences between estimated and known age (bias) being less than one year and average absolute differences between estimated and known age (inaccuracy) being less than six years. Further, individuals in their 90s had bias and inaccuracy measures of less than seven and four years for the quadrants and octants methods, respectively. Such accuracy in age estimation, even into the tenth decade of life, demonstrates that this new method for histological aging considerably outperforms more traditional macroscopic methods of aging in older individuals. Considering increasing life expectancies, this research has great promise in providing forensic anthropologists with a tool to accurately age elderly individuals.

Age Estimation, Skeletal Histology, Forensic Anthropology

A83 Increasing Precision in Age Estimation From the Female Os Pubis: A Composite Technique With >80% Accuracy to Within Ten Years of Actual Age

Janamarie Truesdell, MSc, University of Oxford, School of Anthropology and Museum Ethnography, 51/53 Banbury Road, Oxford, Oxfordshire OX2 6PE, UNITED KINGDOM; Andreas Duering, MA, MS, Oxford University, School of Archaeology, 36 Beaumont Street, Oxford, Oxfordshire OX1 2PG, UNITED KINGDOM; and Nicholas Márquez-Grant, PhD, Cranfield University, Cranfield Forensic Institute, Defence Academy of the United Kingdom, Shrivenham SN6 8LA, UNITED KINGDOM*

After attending this presentation, attendees will be familiar with the implementation of a new, continuum-based technique for estimating age from the female os pubis exhibiting 83.9% precision to within ten years of actual age (Author: 96.5%, Observer 1 (Ob1): 82%, Ob2: 74.9%, Ob3: 82.2%) and a further 67.7% precision to within five years of actual age (Author: 85.9%, Ob1: 56%, Ob2: 66.7%, Ob3: 62.2%).

This presentation will impact the forensic science community by introducing a highly effective, supplementary aging technique to be employed alongside those already in regular use for the estimation of age from the pubic symphysis. Additionally, as it is Computed Tomography (CT) based, this presentation will also add to the growing body of literature advocating for the adoption of proactive medical imaging into biological profiling research, as well as to that of age estimation in the living.

Age estimation from the pubic symphysis continues to be one of the most frequently researched and innovated topics in the field of forensic anthropology. Historically, methodologies have fallen into two distinct paradigms: phased, archetypal "picture-matching" techniques versus equation and weighted variable-based component systems. The methodology introduced by this presentation seeks to bridge this gap by combining the traditional, user-friendly "picture-matching" approach with both component and numbered variable elements and by placing morphological change along a continuum, allowing for variation as well as differences in individual senescence.

The proposed technique, the Truesdell Composite Method (TCM), was developed with the assistance of a mixed British sample of 585 female volunteers, all with verified ages (16 years to 93 years of age) and detailed life histories (gathered by this study in a series of face-to-face interviews cross-referenced with hospital records). In addition to parity, potentially confounding variables such as height, weight, race, activity level, diet, alcohol consumption, tobacco use, medications, infection and/or disease in or around the pelvic area, osteoarthritis and/or osteoporosis, hormone supplementation, and the use of birth control were also taken into account (though none bore any significance on accuracy). For comparison, both left and right pubic bones (volume rendered from CT) were assessed for each individual using the Suchey-Brooks Method for aging the os pubis, the Hartnett Method, and the TCM.[1,2]

Subjects were placed into appropriate Suchey-Brooks ranges an average of 89.4% of the time (86.3%, author excluded), but only 49.4% (49.9%) of the time was the mean within a decade of the actual age and, within that, only 27% (26.9%) of the time was it within five years of the actual age. This is likely a reflection of the sample's propensity toward older individuals (~50% over 65 years). The Hartnett Method fared less well overall, with subjects being placed into appropriate ranges 75.5% (70.4%) of the time but, within the ranges themselves, fared slightly better than Suchey-Brooks with means within ten years of actual age 65.4% (59.6%) of the time and 39.1% (33.2%) within five years. When using the TCM, estimations to within ten years of actual age increased to 83.9% (79.7%) and to 67.7% (61.6%) within five years. For Suchey-Brooks, this constitutes a 34.5% (29.8%) increase in precision to within ten years of the actual age and a 40.7% (34.7%) increase in precision to within five years. For the Hartnett Method, precision to within ten years was increased by 18.5% (20.1%) and to 28.6% (28.4%) within five years.

The proposed technique was not designed to replace established methodologies, whose reliability and efficacy are not in question. Instead, by bringing together the best and most effective aspects of two disparate paradigms, it seeks simply to increase precision, and therefore practitioner confidence (especially regarding older individuals), within the existing framework of the methodologies themselves.

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Age Estimation, Pubic Symphysis, Medical Imaging

A84 Apophyseal Ossification of the Iliac Crest in Forensic Age Estimation: New Standards for Modern Australian Subadults Using Computed Tomography

Nicolene Lottering, BS, Queensland University of Technology, School of Biomed Sci, Faculty of Health, 2 George Street, Gardens Point, Brisbane, Queensland 4001, AUSTRALIA; Mikaela S. Reynolds, MSc, Level 5 Q Block, 2 George Street, Gardens Point, Brisbane, Queensland 4001, AUSTRALIA; Donna M. MacGregor, MSc, Queensland University of Technology, School of Biomedical Sciences, Faculty of Health, Gardens Point Campus, Brisbane, Queensland 4001, AUSTRALIA; Maree T. Izatt, Queensland University of Technology, 2 George Street, GPO Box 2434, Brisbane, Queensland 4000, AUSTRALIA; Caroline Grant, PhD, Queensland University of Technology, Paediatric Spine Research Group, O Block, Level 4, Rm 413, Gardens Point Campus, Brisbane 4001, AUSTRALIA; Clayton Adam, PhD, Queensland University of Technology, Paediatric Spine Research Group, O Block, Level 4, Rm 413, Gardens Point Campus, Brisbane 4001, AUSTRALIA; and Laura S. Gregory, PhD, Queensland University of Technology, School of Biomedical Sciences, Gardens Point Campus, Brisbane, Queensland 4001, AUSTRALIA*

After attending this presentation, attendees will: (1) appreciate recalibrated population-specific age standards for Australian subadults based on the Risser Sign, and the implication of idiopathic scoliosis on the derivation of age estimates; and, (2) become familiar with ontogeny of the iliac crest apophysis using 3D reconstructions of Multi-Slice Computed Tomography (MSCT) data.

This presentation will impact the forensic science community by demonstrating the potential of a contemporary MSCT database of abdomino-pelvic scans to assess the currency of methods such as the Risser Sign for clinical age assessment of maturation milestones.[1] This presentation discusses and surmounts limitations associated with conventional radiographs by utilizing MSCT Multi-Planar Reformatted (MPR) and Volume Rendered Reconstructions (VRR) to formulate Australian standards for forensic age estimation, based on excursion and fusion of the iliac crest apophysis.

Accurate age-at-death estimation of skeletal remains represents a key element in forensic anthropology, while age estimates of living individuals are of increasing importance for forensic medicine, considering the increase in transnational migratory movements. The age of criminal responsibility under Australian federal law is ten years of age, while *doli incapax*, the maximum age of presumption against criminal responsibility constitutes 14 years of age. Wittschieber et al. contend that the Risser Sign is suitable for forensic age estimation, especially the demarcation of the 14th year of life.[2] Combined with other roentgenographic indices of maturation, excursion of the iliac crest is used to estimate remaining growth potential and the likelihood of progression in patients with adolescent idiopathic scoliosis, which influences clinical intervention decisions such as bracing or surgery.

The present study seeks to determine whether the Risser Sign, used routinely for assessing iliac crest maturity in scoliosis patients, is suitable for age estimation of subadults, particularly in cases claiming *doli incapax*. The sample composes MSCT abdomino-pelvic Digital Imaging and Communications in Medicine (DICOM) data (0.5mm/0.3mm) acquired from 255 'trauma-screened' Australian individuals aged 6-25 years, admitted to Brisbane children's hospitals between 2007 and 2014. The Risser US six-stage system was employed to score ossification of the iliac crest. Transition analysis was applied to elucidate maximum likelihood estimates between maturational states; robust age parameters were established using a Bayesian statistical approach, with an MCMC sampler. Volume averaging reconstructions of DICOM datasets, using a coronal reformat were employed to create pseudoradiographs for Risser scoring of trauma-screened children. Standards for Queensland idiopathic scoliosis patients (females: 436, males: 95) aged 6 years to 23 years were derived from clinical databases comprising conventional surveillance radiographs, including the pelvis and analytic data (e.g., Risser Sign, Cobb Angle) from a scoliosis progression study performed by the Paediatric Spine Research Group between 1995 and 2007. Comparisons of maximum likelihood estimates demonstrate no significant developmental anomalies in iliac crest maturation associated with idiopathic scoliosis. Age-at-transition for apophyseal appearance corresponds to 12.99±1.3 years in females and 13.87±0.94 years in males. Posterior distributions signify complete appositional growth (Risser 4) at 15.06 (95% CI:13.5-16.7) years and 15.99 (95% CI:14.9-17.0) years in females and males, respectively, an important demarcation stage for scoliosis management. Lack of discriminant power between stages 2-4 demonstrate that the 14th-year legal demarcation cannot reliably be determined in females using this method on conventional radiographs.

Risser grading on MPR and VRR models of Australian subadults reveals interesting anatomical deviations, highlighting flaws in the ossification progression stated by Risser. Appearance of the apophysis is witnessed 6 months to 12 months earlier in MSCT than pseudoradiographs. Circumventing radiographic limitations such as superimposition, a modified eight-stage MSCT scoring-tier was developed for appearance and fusion of the apophysis, demonstrating origins from three secondary ossification centers. Complete fusion/obliteration occurs between 18.4 years to 19.7 years in males and 19.3 years to 20.3 years in females; indicating secular change in Australian children in contrast to anthropological standards of Coimbra individuals and the 23-year demarcation by Webb and Suchey.[3,4]

The contributions of this original research are extensive. Caution in the derivation of ossification standards from conventional radiographs is advised, with conflicting timings and morphological progression to MSCT assessment. Retrospective clinical data acquisition provides the ideal catalyst for the advancement of anthropological subadult research, demonstrated by the construction of refined, Australian standards for age estimation of the current milieu. Bayesian posteriors of the MSCT scoring-tier demonstrate successful *doli incapax* age estimation for utility in criminal proceedings.

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Age Estimation, Subadult, Iliac Crest Apophysis

A85 DNA Methylation Markers as a Novel Tool for Age-at-Death Estimation in Teeth

Sara C. Zapico, PhD, Smithsonian Institution, Dept of Anthropology, NMNH, MRC 112, 10th & Constitution Avenue, NW, Washington, DC 20560; Bram Bekaert, PhD, University of Leuven, University Hospitals Leuven, Dept of Forensic Medicine, Leuven, BELGIUM; Aubeline Kamalandua, MS, University of Leuven, Forensic Biomedical Sciences, Department of Imaging and Pathology, Leuven 3300, BELGIUM; Wim Van de Voorde, MD, University of Leuven, University Hospitals Leuven, Dept of Forensic Medicine, Leuven, BELGIUM; and Ronny Decorte, PhD, University of Leuven, University Hospitals Leuven, Dept of Forensic Medicine, Leuven, BELGIUM*

After attending this presentation, attendees will consider the possibility of using DNA methylation markers for age-at-death estimation in teeth as an alternative to classical anthropological methods.

This presentation will impact the forensic science community by introducing a novel, innovative, and accurate approach for age-at-death estimation based on the current state-of-the-art research on aging.

Age estimation represents one of the fundamental parameters in forensic anthropology in creating the biological profile toward the correct identification of an individual. This parameter is particularly important in mass disaster scenarios where skeletons are often incomplete, which makes the correct identification of the victims difficult. Teeth are frequently preserved long after all other tissues have disappeared and are often used to estimate characteristics like age at death.

There are several approaches to age estimation based on dental development. In forensic anthropology, the Lamendin technique and its variants are non-invasive methods of age-at-death estimation; however, these methods can only be applied to single-rooted teeth and their accuracy is not guaranteed due to differences in population-specific references. New methodologies for age estimation are based on the natural process of aging, which causes alterations of tissues and organs on different biochemical levels. Recently, it has been discovered that one of these alterations are changes in DNA methylation patterns. In fact, some studies identified and correlated DNA methylation biomarkers with age in blood samples. Although these studies were mainly developed in blood samples, these are potentially interesting in forensic identification because they could help to improve the estimation of age at death.

Since teeth are the hardest tissues of the human body and one of the most abundant types of biological remains available in forensic cases, the goal of this study is to evaluate the potential usefulness of DNA methylation biomarkers for age-at-death estimation in dentin and assess the reliability and accuracy of this methodology in this tissue.

Twenty-nine healthy erupted third molars were collected from dental clinics in Spain (aged 19 years to 70 years). The Smithsonian Institution's ethical committee approved all procedures related to experimentation with human subjects. The teeth were cleaned and the enamel and cementum removed. The dentin was isolated, mechanically ground, and divided in aliquots of 200mg each. Then dentin was submitted for DNA extraction and quantification; 200ng of genomic DNA was bisulfite converted and later amplified by Polymerase Chain Reaction (PCR) for the following genes: ASPA, PDE4C, EDARADD, and ELOVL2. To analyze DNA methylation levels of five CpG sites in these genes, pyrosequencing was performed.

After analyzing pyrosequencing results, a multivariate linear regression model was selected from all methylation sites present in the pyrosequencing assays of ASPA, PDE4C, ELOVL4, and EDARADD by using the step function in R, which selects the model that explains most of the observed variance, predicting age with an adjusted R2 of 0.74 and a Mean Absolute Deviation (MAD) of 4.84 years (p-value <0.001).

This research is the first to explore age-associated methylation in teeth. The findings from this study provide a new quantitative tool for estimating age at death which, in combination with traditional age markers, could improve identification accuracy in forensic cases. Future research may be able to expand on these results, identifying new markers through whole genome CpG studies, using different types of teeth and extending the age range.

Age-at-Death, DNA Methylation, CpG Marker

A86 A Novel Method for Recording Palate Shape in the Estimation of Ancestry

Christopher A. Maier, MA, University of Nevada, Reno, 1664 N Virginia Street, Ansari Business 512, Reno, NV 89557*

After attending this presentation, attendees will learn a new method for scoring palate shape with standardized descriptions. This new method can be used in conjunction with other methods of ancestry estimation to refine the biological profile. Additionally, attendees will gain an understanding of the utility of palate shape as an indicator of ancestry.

This presentation will impact the forensic science community by expanding the traits available for the forensic assessment of ancestry and by providing a standardized means by which to assess palate shape, a trait traditionally scored based on shape variables with somewhat misleading names.

Palate shape has been in use as an indicator of ancestry since 1931 when Hooton included it on the Harvard Blanks list of traits.[1] Since then, it has been included in multiple lists on ancestry-related traits.[2-5] Although a well-established trait, no definition exists of what precisely should be assessed, and there is little agreement as to what shapes are associated with which ancestries. Hooton referred to White, Black, and Asian/Native American palates as pinched, narrow, and wide, respectively, while Krogman and Işcan refer to the palates of the same groups as narrow, wide, and intermediate, and Gill calls them parabolic, hyperbolic, and elliptical.[1-4] The subjectivity inherent in recording this trait has left some practitioners questioning the utility of palate shape as an effective indicator of ancestry.[6] Previous work found that by using digital representations of the palates, individuals were assigned to the correct ancestry 68% of the time, which is more than twice as good as chance; however, the methods outlined in that study require the use of a digitizer and statistical software not available to all forensic anthropologists.

The present study defines a novel rank scale to assess palate shape. For this research, palate shape is defined as the overall trend in shape of both the dental arcade and the underlying alveolar bone and was recorded according to five ordinal character states. States 1, 3, and 5 correspond to the traditional elliptical, parabolic, and hyperbolic shapes, respectively, while states 2 and 4 represent transitional shapes. This newly defined method was then tested on a sample of individuals of known ancestry (n=146).

Data were collected on individuals housed at the Pima County Office of the Medical Examiner, the Donated Collection at the Forensic Anthropology Center at Texas State, and the Donated Collection curated by the Forensic Anthropology and Computer Enhancement Services (FACES) lab at Louisiana State University. Palate shape was recorded on individuals of White, Black, Asian/Native American, and Hispanic ancestry using this new method. Intra-observer error was assessed using weighted Cohen's Kappa (K=0.55, p<0.001), which indicates that there is moderately good agreement as outlined by Landis and Koch, and that this agreement score is significantly different from that expected from chance.[7] A frequency table of the distribution of each score by ancestry was created, and Chi-square analysis indicated that significant differences in palate shape exist between ancestry groups (χ^2=59.9974, p<0.001). A post-hoc test of the Chi-square results indicated that all pair-wise comparisons of ancestry groups were significantly different (p<0.05) with the exception of the Black vs. Asian/Native American comparison (p=0.1282), which was likely not significant due to insufficient sample size.

The adoption of this scale for recording palate shape has initially shown that it is successful at distinguishing ancestral groups and can be useful as a skeletal indicator of ancestry. The use of this scale will provide a standard means of recording palate shape that does not require the use of special equipment, therefore making it useful for a broader range of anthropologists. This new scoring method can then be added to the suite of methods available to forensic anthropologists to estimate ancestry.

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Forensic Anthropology, Ancestry, Macromorphoscopics

A87 Ancestral Variation in Orbital Rim Shape: A 3D Pilot Study

Katie M. Rubin, MS, CA Pound Human ID Laboratory, PO Box 103615, Gainesville, FL 32610; and Valerie DeLeon, PhD, University of Florida, Dept of Anthropology, Turlington Hall, Rm 1112, Gainesville, FL 32611*

After attending this presentation, attendees will better understand how curviplanar relationships along the orbital rim may inform ancestry analyses and may begin to evaluate orbital shape in 3D.

This presentation will impact the forensic science community by highlighting the need to investigate non-metric traits more rigorously as forensic anthropology moves into the future.

Traditional non-metric methods of ancestry assessment posit that orbital shape can be used to help discriminate among broad ancestral groups.[1,2] These claims are based upon visual assessment of crania (rendering them prone to high inter- and intra-observer error) and are supported by limited frequency data. Recently, a handful of studies have used geometric morphometrics to reassess ancestral differences in 2D orbital shape.[3,4] A study by Urbanová assessed ancestral variation in orbital shape in three dimensions; however, this study looked at differences between Czech and Portuguese populations only.[5] To this study's knowledge, no published studies assess 3D orbital variation for the three broad ancestral groups commonly discussed in United States ancestry assessment literature. The results of this pilot study suggest a need to fill this research gap, especially as the demand for forensic scientists to offer statistical support for their methods increases.

The cranial sample used in this study is part of the evidentiary collection of the C.A. Pound Human Identification Laboratory at the University of Florida. The study sample consists of 9 individuals of primarily Asian ancestry (broadly defined), 10 individuals of primarily African ancestry, and 12 individuals of primarily European ancestry.

All crania were digitized using a MicroScribe®. Bregma, nasospinale, and staphylion were digitized to establish a homologous midline plane. Each orbital rim was divided into upper (vault) and lower (facial) curves using dacryon and frontomalare orbitale as homologous start and end points and digitized using the MicroScribe® scan setting. To minimize intra-observer error, each curve was digitized three times. Curve coordinates for each trial were resampled (10 semilandmarks for the upper curve; 15 for the lower) and subjected to sliding semilandmark analysis within specimens using IMP8 Simple3D ChainMan3D executable software. The mean orbital shape for each specimen was calculated with Simple3D. All mean shapes were re-slid together in ChainMan3D to minimize arbitrary differences among specimens. The final configurations underwent generalized Procrustes analysis. Principal Components Analysis (PCA) was conducted using IMP8 ThreeDPCA8, which generates Principal Components (PCs) based on partial warp scores.

A one-way Multivariate Analysis of Variance (MANOVA) was used to assess the effect of ancestry on the first eight PCs, which account for more than 80% of the total inter-individual variation in orbital shape (p=0.0003). The results of the MANOVA appear to be driven by the first two PCs (47.2% of total variation). Post hoc ANOVAs indicated a significant effect of ancestry on PC1 (p<0.0001) and PC2 (p=0.0026). PC3 through PC8 did not provide any additional discrimination. Centroid size did not differ significantly between the ancestral groups and did not have a significant effect on either PC1 or PC2 scores. Differences among groups are driven primarily by curviplanar relationships between contralateral orbital rim margins.

"European" orbits are distinguished from both "African" and "Asian" orbits along PC1. "European" orbits display more marked folding of the orbit in the sagittal plane; the lateral and medial orbital margins extend further posteriorly relative to the superior margin, and the superior orbital rim projects more anteriorly relative to the inferior border.

In contrast, "African" and "Asian" orbits are distinguished from each other along PC2, which describes relationships between the lateral and medial orbital margins. The lateral margin of "African" orbits lies further posteriorly relative to the medial margin when compared to the relatively co-planar "Asian" orbital rims.

The traditional anterior view of the orbits shows apparent differences based on ancestry; however, these are strongly influenced by the curviplanar relationships described above and prone to error based on orientation of the skull.

The study is severely limited by the size and nature of the sample analyzed, but may help guide future studies of a similar nature. Together, this study and that of Urbanová suggest that curviplanar relationships may be the most ancestrally informative aspect of orbital rim shape. If future studies address the limitations of these studies, they may provide valuable insight into, and statistical support for, the use of non-metric ancestry assessment in forensic analyses.

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Ancestry, Orbit, Morphometrics

A88 Missing Data Imputation Methods Using Morphoscopic Traits and Their Performance in the Estimation of Ancestry

Michael W. Kenyhercz, PhD, University of Tennessee, 250 S Stadium Hall, Knoxville, TN 37996; Nicholas V. Passalacqua, PhD, 1559 Mount Vernon, East Lansing, MI 48823; and Joseph T. Hefner, PhD, Michigan State University, Dept of Anthropology, 355 Baker Hall, East Lansing, MI 48824*

After attending this presentation, attendees will understand the effects of missing data imputation on the estimation of ancestry from the human cranium using morphoscopic variables.

This presentation will impact the forensic science community by demonstrating which missing data imputation methods have the highest accuracy when working with morphoscopic traits for the determination of ancestry.

Missing data is an inherent problem in biological anthropology due to the fragile nature of osseous material; these issues are compounded in forensic anthropology as remains in forensic contexts are often subjected to peri-mortem trauma or taphonomic alterations that damage or destroy bony morphology. Often in cases with missing data, the variables in question are just excluded from analysis; however, the avoidance of analytical processes when missing data are present have the potential to significantly decrease the ability to reliably estimate aspects of the biological profile, limiting the number of variables used or decreasing the power of the estimations.

While different methods function differently, the goal of missing data imputation methods is to accurately estimate the missing values, using the other observed values. Unfortunately, the analysis of datasets with missing values receives little attention and listwise deletion is the most common form of handling cases with missing values; however, this is often not possible in forensic anthropology, where the entire sample is a single case that may present varying levels of missing data. Because of this, the ability to accurately impute missing data in forensic anthropology is paramount. The goal of this project is to quantify the accuracy of morphoscopic data in conditions with moderate (25%) and severe (50%) amounts of missing data.

Four data imputation techniques were selected to examine which of the missing data imputation methods performed best: Hot-Deck, Iterative Robust Model (IRMI), K nearest-neighbor (kNN=5), and the variable medians. A subset of Hefner's Macromorphiscopic Databank was used. The full sample consisted of 688 individuals from three population groups (Black=292, Hispanic=186, and White=210). Six commonly used cranial macroscopic variants were scored in accordance with Hefner, and Hefner and Ousley.[1,2] Two versions of the dataset were then created wherein values were randomly deleted from each variable so that 25% and 50% of the data were considered missing. The same data subsets were used for each of the imputation techniques, and the efficacy of each technique was based on absolute agreement using the Intra-Class Coefficient (ICC). Correct classification rates and Mahalanobis D^2 values were calculated for the original dataset with actual measures and each of the imputed datasets in order to examine the effects of imputed data on biodistance and classification. Results suggest that Hot-Deck imputation is the most accurate method with 25% missing data, with this imputation method consistently performing at the highest levels of agreement (up to ICC=0.97), with the least impact to the D^2 and, ultimately, classification. In severe instances of missing data (50%), IRMI consistently produced the highest levels of agreement. Kenyhercz and Passalacqua examined the effects of missing data using metric cranial data and found that when dealing with continuous variables, the kNN imputation method had the best performance in both moderate (25%) and severe (50%) missing data conditions.[3]

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Non-Metric Data, Imputation, Missing Data

A89 Skeletal Sex Estimation in a Modern Cuban Sample

Meredith L. Tise, PhD, University of Lincoln, School of Life Sciences, Brayford Pool, Lincoln LN6 7TS, UNITED KINGDOM*

After attending this presentation, attendees will understand which skeletal elements and standard measurements of the cranium and postcranial skeleton are the most accurate when estimating the sex of modern Cuban individuals in a forensic anthropological context.

This presentation will impact the forensic science community by offering new techniques when analyzing skeletal remains that have the potential to be an individual from Cuba, most specifically when metrically estimating sex. This presentation will also incorporate the significance of this research with the recent political and economic changes between the United States and Cuba.

Craniometric and postcranial metric data were collected from 111 known modern Cuban individuals located at the Museo Antropológico Montane at the University of Havana in Havana, Cuba. This sample contains data from 65 male and 46 female individuals. Multivariate and univariate statistical analyses, including Discriminant Function Analyses (DFA), were conducted in SAS® 9.4 to establish classification functions and sectioning points with associated classification rates.

Out of the 27 standard cranial measurements collected with a MicroScribe® G2 digitizer, eight measurements were separated using a stepwise selection procedure to include GOL, XCB, ZYB, WFB, AUB, OBH, PAC, and MOW. Based on these eight cranial measurements, and the resulting classification function, females were correctly classified 86.05% of the time and males were correctly classified 87.50% of the time, based on the cross-validation classification rates.

Forty-one standard postcranial measurements were collected from the long bones of each individual, including the humerus, radius, ulna, femur, tibia, and fibula. The stepwise selection procedure selected 12 measurements that are the most accurate when estimating the sex of these long bones: HUMXLN, HUMEBR, RADXLN, RDH, RADAPD, RADTVD, ULNXLN, ORL, BCB, FEMEBR, TIBXLN, and TIBNFX. Based on the multivariate analyses, the humerus resulted in the highest overall cross-validation classification rate of 96.93%, followed by the radius with 92.17%. When assessing each stepwise selected measurement individually, the Humerus Epicondylar Breadth (HUMEBR) resulted in the highest cross-validation rate of 96.93%, followed by the ulna Olec-Radial Notch (ORL) with a classification rate of 90.97%. These results demonstrate a high accuracy for utilizing cranial and postcranial metric analyses with estimating the sex of the skeletal remains of Cuban individuals.

Not only will these skeletal sex estimation techniques be beneficial to forensic anthropologists in Cuba, forensic anthropologists practicing in the United States can also utilize the results of this research. According to the 2010 United States Census, the Cuban population increased by 44% in the United States from 2000 to 2010, with an increase from 1.2 to 1.8 million within those ten years.[1] In 2010, approximately 77% of Cuban individuals in the United States resided in the southeastern states, with the majority of these individuals living in Florida.[1] Therefore, forensic anthropologists conducting casework in Florida are most likely to be confronted with the need for methods derived from Cuban individuals.[2] Recent studies have demonstrated the difficulty and inaccuracy of using methods derived in the United States on Cuban individuals, primarily as a result of the differential population history Cuba has experienced since Spanish colonization took place in the late 1400s.[3,4] With the recent advent of diplomatic relations being restored between Cuba and the United States, collaborations between the two countries will begin to excel, including in the forensic science community.

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Cuba, Sex Estimation, Metrics

A90 Sexual Dimorphism of the Radial Tuberosity: Geometric Morphometric Approach With a Structured-Light 3D Scanning System

Go-Un Jung, BS, Yang-choun Gu, Mok-5-Dong, 911-1 bun-ji, Ewha Womans Hospital, Uwi-hak-GuanA dong, 511 ho, Seoul 158-710, SOUTH KOREA; Byoung-Ha Kim, BS, Academy for Human Modeling, BM Art Center, Hapjeong-dong, 377-14, Mapo-gu, Seoul 121885, SOUTH KOREA; U-Young Lee, MD, The Catholic Univ of Korea, Dept of Anatomy, Coll of Med, 505, Banpo-dong, Seocho-gu, Seoul 137701, SOUTH KOREA; Deog-Im Kim, PhD, Keimyung University, College of Nursing, 1095 Dalgubeol-daero, Dalseo-gu, Daegu 704701, SOUTH KOREA; Dae-Kyoon Park, MD, PhD, Soonchunhyang University, Department of Anatomy, College of Medicine, 31 Sooncheonhyang 6-gil, Dongnam-gu, Cheonan-si, Seoul 31151, SOUTH KOREA; and Yi-Suk Kim, MD, PhD, Ewha Womans University, Dept of Anatomy, School of Medicine, 911-1, Mok5-dong, Yangcheon-gu, Seoul 158710, SOUTH KOREA*

WITHDRAWN

*Presenting Author

A91 Cranial Morphological Sexing Trait Patterns Differ Across Populations

Monica M. Thompson, University of La Verne, 1950 Third Street, La Verne, CA 91750; Kaitlyn A. Lopez*, University of La Verne, 1950 Third Street, La Verne, CA 91750; and Kanya Godde, PhD*, 1950 Third Street, La Verne, CA 91750*

After attending this presentation, attendees will be aware of the similarities and differences in expression of four cranial morphological sexing traits between Egyptians and modern Americans. Further, attendees will understand the implications of these differences when applying the cranial morphological sexing technique, which could lead to an increase in the accuracy of estimating sex.

This presentation will impact the forensic science community by highlighting the limitations of the cranial morphological sexing method and showing how it can be applied successfully by knowing the morphological patterns specific to individual populations.

When applying the cranial morphological sexing technique, biological anthropologists adjust the method to suit the population under study. Population patterns in trait expression have not been formally published for testing and quantification in a forensic context. Thus, an evaluation of the different degrees to which cranial morphological traits are expressed across populations is necessary. The hypothesis this research tested was that traits of the cranial morphological sexing method differ in magnitude between modern Americans and Egyptians.

Two populations (N=457) were analyzed for each of the five major cranial morphological sexing traits: glabella, mastoid process, supraorbital margin, nuchal crest, and mental eminence. Modern Americans from two collections with documented sex, the William M. Bass Donated Skeletal Collection (Bass Collection) and Hamann-Todd, were observed on a scale of one through five for each of the five characteristics. The modern Americans represent inhabitants of rural and urban areas born during the past 186 years. The Egyptian sample is comprised of observations made on individuals from four sites, spanning Upper and Lower Egypt and from the Predyanstic through the New Kingdom time periods. The Egyptian collection is curated in the Phoebe A. Hearst Museum of Anthropology at the University of California, Berkeley (UCB).

As the Egyptian sample had few associated pelvises to verify sex, cranial measurements from the Giza Egyptian sample in the Howell's data set were input into discriminant analyses to create a discriminant function for an estimate of sex on the UCB Egyptian sample. The result was 97% accuracy of Howell's measurements against Howell's assigned sex. This population-specific discriminant function from Giza was then applied to the UCB Egyptian sample to estimate sex from cranial measurements. Only skulls with 80%-and-above accuracy rates from the discriminant function were retained.

A two-tailed Fisher's exact test with the Freeman and Halton adaptation for RxC tables was executed on the data categorized by sex (documented or established by the discriminant analysis described above), population, and degree of expression per trait. Mental eminence was not assessed as few mandibles were preserved in the Egyptian sample and expression of mental eminence was obscured by dental disease or edentulism in many of the modern Americans from the Bass Collection. Eight cross-tabulation tables were generated, while sex was controlled, to assess population and degree of expression. Of these eight analyses, only one was not significant at α=0.10 or higher (glabella in females), while the other results were all significant at the α=.01 level. Thus, in general, the null hypothesis can be rejected; the probability a particular sex will exhibit the same degrees of expression regardless of population affiliation is not supported. In this study, the degree of expression of these traits is population specific. It can also be concluded from the cross-tabulation tables that modern Americans tend to have a higher degree of expression of these traits than Egyptians.

The results demonstrate that these four traits cannot be universally applied for estimating sex from the cranium. Hence, forensic practitioners should be aware that population specificity ought to be factored into the application of this method. As regards the American and Egyptian populations, forensic anthropologists should anticipate that higher trait scores (e.g., five in males and three in females) are more frequent in modern Americans and that this is not solely attributable to sex.

This research was supported by a William M. Bass Endowment award and a University of La Verne Academy grant.

Modern Americans, Population Specific, Discriminant Analysis

A92 An Analysis of Sexual Dimorphism Using Geometric Morphometrics (GM) of the Femur and Tibia: The Use of GM in Assessing Sex of Fragmented Remains

Amanda K. Costello, MS, 511 Bienveneda Avenue, Pacific Palisades, CA 90272*

After attending this presentation, attendees will have a better understanding of the usability of GM to accurately assess sex of unidentified remains. Attendees will also be educated on which areas of two skeletal elements — the femur and tibia — preliminarily indicate they are more indicative of sex-dependent size and shape variation when compared to others.

This presentation will impact the forensic science community by providing a method for assessing sex of fragmented remains when other methods are not possible, which has the potential to provide the researcher with critical forensic information that may not otherwise be attainable.

Biological anthropologists have recently been utilizing GM to investigate sexual dimorphism among modern *Homo sapiens*. To analyze sexual dimorphism of the femur and tibia using GM, landmark data were registered using a MicroScribe® on 250 individuals of known sex and age at death from the William M. Bass Donated Skeletal Collection. The sample was limited to individuals of "White" ancestry in order to eliminate population bias. A combination of landmarks and semi-landmarks were collected on the proximal and distal epiphyses of each bone, which captured the overall size and shape variation present in the sample. Classification rates for males (ages 19 years to 96 years) and females (ages 29 years to 97 years) for the proximal femur were 80.8% and 78.4%, respectively; for the distal femur, 92.6% and 89.6%, respectively; for the proximal tibia, 80.8% and 83.2%, respectively; and for the distal tibia, 81.6% and 80.8%, respectively.

This study indicates the knee joint is the most dimorphic, followed by the ankle, then the hip. The results are similar to other studies that indicate the knee is more sexually dimorphic, though here it was found the distal femur was more dimorphic when compared to the proximal tibia. This preliminary research indicates that in comparison to standard measurements, GM may provide a more reliable method for sex estimation when used on the knee. Further research applications excluded landmarks to determine the usability of the method if fragmented remains are present due to taphonomic processes, such as the case may be in forensic circumstances. When landmarks were excluded simulating taphonomic damage, the distal femur still presented the highest classification rates, averaging more than 81% for males and females, followed by the distal tibia, averaging more than 73% for males and females, followed by the proximal tibia, averaging more than 71% for males and females, and the epiphysis with the lowest classification rate after landmark removal was the proximal femur, averaging more than 63% for males and females.

This application revealed which areas of the femur and tibia are more indicative of sex-dependent size and shape variation when this method was applied to a modern "White" population. These areas are controversial in that they are not areas previously associated with sexual dimorphism. Knowledge of these areas will change how future research analyzing sexual dimorphism of the skeletal elements of the leg is conducted.

Sexual Dimorphism, Geometric Morphometrics, Forensic Anthropology

A93 Metric Assessment of the Pubic Bone to Determine the Accuracy of Known and Novel Data Points for Sex Estimation

Kathleen A.S. Blake, PhD, State University of NY at Oswego, Dept of Anthropology, Mahar 441, Oswego, NY 13126; Hallie Gaffney*, State University of New York at Oswego, Mahar Hall, Anthroplogy Dept, Oswego, NY 13126; and Kristen Hartnett-McCann, PhD*, Office of the Chief Medical Examiner, Forensic Anthropology, 11 Shuttle Road, Farmington, CT 06032*

After attending this presentation, attendees will understand the variation of 13 metric and 3 non-metric traits on the pubic bone from an adult skeletal sample with known demographic data.

This presentation will impact the forensic science community by: (1) evaluating pubic bone dimorphism between adult males and females from metric and non-metric traits; (2) providing landmarks and measurements that demonstrate reduced error and increased reliability of sex assessment of the pubic bone to allow for comparison by future researchers; and, (3) assessing novel data points that suggest additional sex determination methodologies than previously used. This presentation adds to the research on reliability and repeatability of forensic anthropological sex determination methods through other analysis of adult skeletal samples with known age and sex compared to previously published rates. Additionally, this research quantifies shape differences in the pubic bone of males and females, adding to the growing list of studies undertaken to meet the rigorous *Daubert* ruling scientific standards.[1]

The determination of the biological sex of skeletal remains is an important early step in forensic and archaeological analyses. Not only does it eliminate roughly half of the population from the search in forensic cases, but components of a biological profile, such as stature, ancestry, and age at death, are based on that initial sex assessment.[2] While distinctions exist between male and female skeletons before birth, the more visible skeletal dissimilarities manifest at puberty, when hormones stimulate secondary sex characteristic development.[3] Anthropologists use the pelvis as it is the most sexually dimorphic element of the human skeleton, both morphologically and metrically, due to functional requirements of both bipedality and safe parturition in females.[4,5] Phenice first recognized that the ventral arc, subpubic concavity, and medial border of the inferior pubic ramus were all highly sexually dimorphic.[6] Moreover, the pubic bone shape can be used to visually assess sex; in females, the body of the pubis is larger and more rectangular, while male pubic bones are somewhat triangular in shape.[7]

This study examines a modern, diverse sample from the Maricopa County Forensic Science Center (FSC) in Phoenix, AZ. A subset of the FSC collection, n=400, (120 females and 280 males) was examined by three observers. All measurements were taken from the left side when available; if damaged or missing, the right side was substituted. A total of 13 measurements from both previously identified and novel points were taken and included height and breadth of face, several measurements of the pubic body from the obturator foramen, and ischiopubic ramus thickness. In addition, three non-metric features were visually evaluated: the ventral arc, the subpubic concavity, and the overall shape of the pubic body.

This research determined that a relationship exists between 12 measurements and biological sex. Between males and females, no significant differences were found in the variances for the measurements taken, with nearly all normally distributed. Independent t-tests showed significant differences in the means for 12 of the 13 measurements, with most at the p=.000 level. All but one measurement showed correlations to sex at either a significance level of 0.01 or 0.05, with pubic body width and perpendicular inferior pubic body width the strongest at .550 and .531, respectively. Non-metric traits also correlated to sex; for example, the ventral arc correlated to sex at the 0.01 significance level with a correlation coefficient of 0.792. Inter-observer reliability was tested using intra-class correlation. For the two most significantly correlated traits, pubic body width and perpendicular inferior pubic body width, both supported the reliability of these measures, with the pubic body width intra-class correlation the highest at 0.962, with a 95% Confidence Interval (CI) (0.954, 0.969).

In forensic anthropology today, sex determination methods must meet forensic legal standards of reliability and repeatability and be developed from samples of known age and sex; however, current methods provide limited accuracy, as many methods are subjective, non-metric, and developed on non-modern skeletal populations.[8] The creation of standardized, repeatable, metric sex determination methods from modern populations is essential. Considerable variation was present in the shape and size of pubic bones in this population skeletal sample and landmark determination was more difficult for some measurements than others; however, once identified, these measurements were reliable and they show promise as metric determinants of sex on the pubis.

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Sex Determination, Pubic Bone, Metric Assessment

A94 An Assessment of Sexual Dimorphism in the Sternal Fourth Rib: A 2D Morphometric Approach

Andrew C. Seidel, MA, Arizona State University, Human Evolution/Social Change, PO Box 872402, Tempe, AZ 85287-2402; and Laura C. Fulginiti, PhD, Forensic Science Center, 701 W Jefferson, Phoenix, AZ 85007*

After attending this presentation, attendees will be informed about the presence of measurable sexual dimorphism in the contours of the superior and inferior margins of the sternal two centimeters of the fourth rib.

This presentation will impact the forensic science community by providing data suggesting that the shape of the sternal end of the fourth rib has the potential to be used in the estimation of sex from isolated ribs. In turn, this finding would allow for the construction of biological profiles for unknown decedents for whom more traditional skeletal indicators of sex (e.g., cranium and os pubis) are damaged or unavailable.

This research was conducted using the Hartnett-Fulginiti collection housed at the Forensic Science Center in Maricopa County, AZ. This collection is comprised of more than 600 specimens of pubic symphyses and associated sternal ends of the fourth ribs from decedents of known sex, age at death, and ancestry. Two hundred individuals (100 males, 100 females) were randomly selected from the collection for analysis. Specimens were positioned so they were level and photographed with a scale. Where possible, left fourth ribs were utilized. When left rib ends were damaged or otherwise unavailable, right rib ends were photographed and the resulting digital image was mirrored in order to match the orientation of the left rib images. Specimens that were damaged or less than 2.5cm in length were removed from the analysis, resulting in a final sample size of 140 individuals (70 males and 70 females) ranging from 18 years to 93 years of age.

Each image in the final sample size was calibrated using the photographed scale and a suite of 40 landmarks were digitized at approximately 1.0mm intervals beginning at the edge of the sternal pit and extending for a length of 2.0cm along the contours of both the superior and inferior margins of the rib. Actual interval markings ranged between 0.97mm and 1.03mm due to software and image resolution limitations. Landmark data were then subjected to a Generalized Procrustes Analysis (GPA) to investigate differences in shape between male and female sternal rib contours. As GPA removes the effects of size, location, and rotation within landmark configurations, it should negate the effects of body-size dimorphism and allow for the direct comparison of sternal rib shape between males and females. The resulting Procrustes coordinates were then subjected to a principal components analysis and component scores were evaluated for evidence of sexual dimorphism.

Results indicate that there are significant differences between males and females in regard to their first Principal Component (PC1) scores (t=11.326, v=138, p-value <2.2e-16). In this analysis, PC1 accounts for 57.8% of the total variation in shape and represents (in females) a constriction of the rib shaft near the sternal end as well as an overall more elongate shape to the sternal 2cm of the fourth rib. In contrast, males typically lack such a sternal constriction and exhibit a broader sternal rib morphology, resulting in a slightly flared appearance. These differences are confirmed by a Discriminant Function Analysis (DFA), the results of which indicate a Procrustes distance between male and female landmark configurations of 0.093 and a Mahalanobis distance of 4.045, both of which are significant (p-value <0.0001 as determined by permutation tests using 1,000 iterations). Moreover, the results of a leave-one-out cross-validation of the DFA resulted in a 21.4% misclassification rate for females and a 22.8% misclassification rate for males. Decreasing the number of utilized landmarks to 30 increased the percentage of total shape variation accounted for by PC1 to 58.9% and resulted in improved misclassification rates produced by cross-validation (17.1% for females and 20.0% for males).

These results suggest that meaningful sexual dimorphism exists in the shape of the sternal fourth rib and that it may be profitably employed in the estimation of sex for unknown decedents, especially in situations in which more commonly used techniques cannot be employed.

Fourth Rib, Sex-Estimation, Morphometrics

A95 A Geometric Morphometric Comparison of Pelvic and Cranial Sexual Dimorphism

Kaleigh C. Best, MS, 2800 W Murphysboro Road, Carbondale, IL 62901; Luis L. Cabo, MS, Mercyhurst University, Dept of Applied Forensic Sciences, 501 E 38th Street, Erie, PA 16546; and Heather M. Garvin, PhD, Mercyhurst University, Dept of Applied Forensic Sciences, 501 E 38th Street, Erie, PA 16546*

After attending this presentation, attendees will understand how sexual size and shape dimorphism compares in the human os coxae and cranium, including which shape changes contribute the most to sex differences.

This presentation will impact the forensic science community by providing cross-validated correct sex classification rates for the os coxae and cranium and demonstrating to forensic anthropologists which of the two elements provides the most reliable sex estimation when case results may be contradictory.

In humans, the os coxae and the cranium are commonly referred to as the most sexually dimorphic regions of the skeleton and thus are often used to estimate the sex of individuals in a variety of physical anthropology subfields, including paleoanthropology, bioarchaeology, and forensic anthropology. Although there are numerous studies analyzing either pelvic or cranial sexual dimorphism, these studies utilize various populations, samples, sample sizes, types of data, methods, or statistical analyses, making a direct comparison between the resultant dimorphism values invalid. Only one study was found in which pelvic and cranial dimorphism was analyzed using a single sample, but it only compared non-metric sex estimation results, which are known to be somewhat subjective. The goal of this study was to use landmark data and geometric morphometric analyses to compare sexual size and shape dimorphism in the os coxae and cranium in a single sample. The use of a single study sample for both analyses means that the obtained sex classification results are directly comparable and will provide forensic anthropologists with information regarding the reliability of these two elements in sex estimation methods.

Forty-two landmarks from the cranium and 12 landmarks from the os coxae were digitized using a MicroScribe® on a sample of 113 United States Black adults (aged 17 years to 70 years) from the Hamann-Todd Osteological Collection. Following a Procrustes superimposition, principle component and discriminant function analyses were used to assess and compare the degree of sexual shape and form (combined shape and size) dimorphism present in both skeletal regions. Univariate analyses were performed to evaluate which specific shape changes were contributing the most to the sex differences. Centroid size was used to assess sexual size dimorphism.

The results of the shape, form, and size analyses all indicate that the os coxae is more sexually dimorphic than the cranium. The discriminant function analysis performed on the os coxae shape variables resulted in a cross-validated correct sex classification rate of 99.1%, compared to 84.1% in the cranium. Including size in the shape analyses (i.e., form) and analyzing size independently (i.e., centroid size) did not increase sex classification rates, indicating that sex differences in these elements occurs primarily in shape. The geometric morphometric analyses confirmed that relative pubis length, sciatic notch breadth, and subpubic concavity were the most important shape variables in pelvic dimorphism. In addition, os coxae height relative to ilium breadth also contributed to sex differences. In the cranium, the geometric morphometric analyses revealed sex differences in facial height, vault breadth, cranial base flexion, nasal width, and glabellar prominence.

As this study uses a single sample to analyze sexual dimorphism in the os coxae and cranium, it eliminates many extraneous variables (e.g., sample and method differences) and allows a direct comparison between the skeletal regions. Results confirm that the os coxae is significantly more dimorphic than the cranium; thus, when assigning sex to an unknown skeleton, forensic anthropologists should rely more heavily on pelvic morphology. Geometric morphometric shape analyses conducted on the os coxae landmark data can discriminate between the sexes with up to 99% accuracy and provide an objective method to quantitatively analyze traditional non-metric sex traits.

Sex Estimation, Os Coxae, Cranium

A96 A Reassessment of Walker Cranial Non-Metric Traits on Undocumented Border Crossers Along the South Texas Border

Brittany S. McClain, BA, Texas State University, 8901 Jesse James Drive, Austin, TX 78748; Cassie E. Skipper, BS, Texas State University, New Braunfels, TX 78130; and Marilyn Isaacks, BA, Texas State University, 15931 Watering Point Drive, San Antonio, TX 78247*

After attending this presentation, attendees will know to proceed with caution when sexing Hispanic crania using Walker visually assessed cranial traits as Hispanic populations do not exhibit the full range of cranial morphological variation assumed in this method.

This presentation will impact the forensic science community by aiding in this humanitarian effort to identify and repatriate these individuals by acknowledging the necessity for population-specific techniques and to make other researchers aware of the potential issues when solely using crania for sex estimation.

The increase in undocumented border crosser deaths in the harsh environments along the South Texas border has created a present humanitarian disaster in which forensic anthropologists must utilize all available skeletal resources, even when little remain. The problem is especially acute in Brooks County, TX, where remote ranchland is abundant, thereby making it easier for migrants to cross the Texas-Mexico border, although it is also more perilous due to weather conditions. Project Operation Identification (OpID) was created at Texas State University in response to the increasing border crosser fatalities. OpID addresses this humanitarian disaster and serves to identify and repatriate the skeletal remains of undocumented border crossers who died crossing the South Texas border.

The unforgiving Texas environment can both limit the recovery of skeletal elements and lead to poor preservation. As a result, OpID utilizes all available skeletal material to create a biological profile. When pelvic skeletal elements are not present to estimate sex, the cranium is used as an alternative and analyzed using the Walker non-metric sexing method, which is based on scoring visually assessed cranial traits.[1] This method has traditionally been used on all populations without dispute when no other appropriate technique is available for the specific population.

It is currently unknown whether Hispanic populations exhibit the full range of cranial morphological variation assumed in the Walker scoring system. The current research serves to test the applicability of the Walker cranial non-metric sexing method to a Hispanic sample and to discern if these individuals express the expected full range of variation. Inter-observer reliability between the three researchers was assessed and confirmed prior to scoring the crania. The OpID crania were seriated based on each of the five traits and scored separately. Twenty-one crania were scored for nuchal crest, mastoid process, supra-orbital margin, and glabella. Because one individual was missing a mandible, only 20 crania were scored for mental eminence.

The results for each trait were analyzed using a logistic regression equation provided by Mercyhurst University to estimate sex, Chi-square goodness of fit, and Cramer's V (0.584, *p*-value=.02).[2] The Chi-square results of this preliminary study show the estimated sex of the OpID individuals based on pelvic morphology is significantly different from the sex estimated using the Walker cranial non-metric method (X2=7.853, df=2, *p*-value=.02); however, there was a strong correlation between sex and the traits scored (Cramer's V=0.584, *p*-value=0.02). Further, the Hispanic sample tested did not exhibit the full range of variation for nuchal crest, supra-orbital margin, and mental eminence described in the Walker article. While the Walker method can be used to estimate sex when only crania are present, researchers should express caution when using this method until the scores are shifted to more accurately represent the Hispanic population.

In conclusion, the sampled Hispanic crania do not exhibit the full range of variation outlined in Walker.[1] To sex the crania of Hispanic individuals, a more appropriate method should be employed and utilized to account for the range of variation exhibited by Hispanic crania. The impact of the present research will aid in this humanitarian effort to identify and repatriate these individuals by acknowledging the necessity for population-specific techniques and to make other researchers aware of the potential issues when solely using crania for sex estimation.

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Undocumented Border Crossers, Sex Estimation, Cranial Non-Metric Traits

A97 Stable Isotope Investigation of Mother-Infant Pairs and the Implication for Forensic Casework

Inga Siebke, Sulgenauweg 40, Bern, SWITZERLAND; Fabian Kanz, PhD, Medical University of Vienna, Department of Forensic Medicine, Sensengasse 2, Vienna, YT 1090; Carsten Witzel, PhD, University of Hildesheim, Marienburger Platz 22, Hildesheim 31141, GERMANY; and Sandra Lösch, PhD, University of Bern, Institute of Forensic Medicine, Dept of Physical Anthropology, Sulgenauweg 40, Bern 3007, SWITZERLAND*

After attending this presentation, attendees will gain: (1) an understanding of the significance of stable isotope analysis and tooth histology in evaluating the survival time of disposed neonates; and, (2) knowledge of the applicability of bioarchaeological research for forensic science.

This presentation will impact the forensic science community by providing results using an innovative approach of stable isotope analysis combined with tooth histology in the context of stillbirth or infanticide. The research will add information to the knowledge of neonatal stable isotope values.

The news frequently reports on forensic investigations in relation to disposed dead infants. The greater the decomposition, the more difficult it becomes to evaluate the cause of death. The main question often raised is whether the neonate was stillborn or was a victim of infanticide. An indication of whether a neonate was born alive is the presence of a Neonatal Line (NNL); however, current research has shown that the NNL is detectable after seven to ten days of survival.[1,2] Stable isotope analysis is frequently used in forensic and archaeological settings and the relationship of the values between mother and infant has been studied.[3-6] In contrast, the question of detecting stillbirth or infanticide has received less attention.

It has been shown that breastfed children exhibit increased δ15N values based on stable isotopes of the hair and fingernails.[3,4] This is not applicable when decomposition is too advanced. Therefore, the goal was to investigate the collagen values of δ15N of mother-infant pairs as neonatal bones are often recovered at forensic scenes.

Three mother-infant pairs with reliable relations from St. Poelten, Austria, were used to evaluate the hypothesis that breastfed neonates express higher δ15N values than their mothers. For the stable isotope analysis, samples of the same skeletal element were taken. Tooth histology was performed if teeth were available. Additionally, 17 human remains from the archaeological site of Petinesca, Switzerland, were analyzed.

The collagen extraction was performed following a modified acid-base extraction method.[7] Isotope ratio mass spectrometry was used for the calculation of the 15N/14N ratio and collagen quality was evaluated. For the tooth histology, a standard protocol was followed, a light microscope and a scanning electron microscope were employed, and micro radiography was performed.[2]

The stabile isotope results of the mother-infant (estimated age: six to ten lunar months) pairs indicate that no breastfeeding signal is present and no NNL was observed. For the second series (estimated age: 8.5 lunar months to 2 months *ex utero*), a breastfeeding signal was observed for all except one individual that exhibited decreased δ15N values compared to the other infants and similar δ15N values to the female average. The tooth histology of this individual revealed no NNL.

It is seen that stable isotope analysis can assist in the evaluation of the survival time of neonates; however, limitations such as the unknown turnover rate of collagen in developing bones require controlled studies. In conclusion, it is believed that stable isotope analysis could become a useful tool for forensic science when dealing with neonatal remains.

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Stable d15N Isotope, Neonatal, Survival Time

A98 Bone Histology Sampling Sites for the Identification of Undocumented Border Crossers Along the United States-Mexico Border

Lauren Alyssa Meckel, BS, Texas State University, 1509 Marlton Street, San Marcos, TX 78666; Sophia Mavroudas, MA, Texas State University, 601 University Drive, ELA 232, San Marcos, TX 78666; Victoria M. Dominguez, MA, Ohio State University, Division of Anatomy, 1645 Neil Avenue, Columbus, OH 43210; and Kate Spradley, PhD, Texas State University, Dept of Anthropology, 601 University Drive, San Marcos, TX 78666*

After attending this presentation, attendees will understand the difficulties of estimating age at death of Undocumented Border Crossers (UBCs) along the United States-Mexico border, and the importance of sampling site choice for histological age estimation.

This presentation will impact the forensic science community by resolving certain issues challenging anthropologists working to identify UBCs in the growing humanitarian crisis along the United States-Mexico border.

The death of UBCs along the United States-Mexico border is an unacknowledged humanitarian crisis currently affecting the United States. The immense skeletal diversity represented within this group presents new challenges in identification as population-specific methods are lacking for this group. Since 2013, the Forensic Anthropology Center at Texas State (FACTS) has been working to identify UBCs from Brooks and surrounding counties in Texas. As part of this effort, FACTS has accepted 78 UBCs from exhumations and from the Webb County, TX, Medical Examiner's Office. Due to the nature of the UBC deaths along the border, anthropologists play a vital role in identifying these individuals and repatriating their remains to grieving families

A critical aspect of UBC identification is an accurate age-at-death estimate to narrow the list of potential matches for repatriation. Previous studies have confirmed that combining both gross morphology and histomorphology age estimation provides a more complete picture of age-related skeletal changes. The goal of this study is to examine which bone histology sampling site is the most appropriate indicator of UBC age at death to help increase identifications and gain a better understanding of skeletal age in UBCs.

The remains of N=29 (15 males and 14 females) UBCs were examined using histological analysis of the femur and of the midshaft of the sixth rib.[1-3] The sex of each individual was determined either by soft tissue or skeletal analysis. Due to sampling constraints, only the anterior femur at the midshaft was sampled and analyzed. Gross morphology estimates were gathered from case reports and age indicators included pubic symphysis, sternal rib ends, and auricular surface.

To determine the most appropriate histological sampling site for UBC identification, the mean age for each site-specific histological method was calculated. Additionally, the femoral and rib age estimates were compared to the gross morphology age range estimates from each individual. Agreement between histology age and gross morphology age was determined by whether the histology point age estimates fell within the gross morphology age estimate ranges. Inter-observer error for each histological method was calculated.

The mean ages for the femur and rib methods were 45.7 years and 37.1 years, respectively. Results show that 2% of the femoral histology point age estimates overlap with the gross morphology age estimates, while 93% of the rib histology point age estimates overlap with the gross morphology age estimates. Inter-observer error was non-significant at $p<0.05$. Compared to the gross morphology mean age (33.7 years), both of the histology methods overaged the sample, but the rib method had a lower inaccuracy. This suggests that with current available methods, the rib is a better sampling site for UBC identification. Overall, the femur method was a poor indicator of UBC skeletal age; however, the remodeling counts of the femur show a positive trend with age (R^2=0.51). This suggests that although this method is not applicable to UBC age-at-death estimation, there is potential for developing new methods using the anterior femur to accurately estimate UBC age at death.

The results of this study indicate that the midshaft of the sixth rib is the most appropriate histological sampling site for UBC identification. The results also suggest that future research focusing on the anterior femur of UBC groups could prove appropriate for UBC identification if new methods are developed with appropriate demographics.

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Histology, Aging, Operation Identification

A99 Estimation of Age-at-Death Using Femoral Cortical Thickness, Biomechanical, and Histological Variables

Megan E. Ingvoldstad, PhD, DPAA Laboratory, 310 Worchester Avenue, Bldg 45, Joint Base Pearl Harbor-Hickam, HI 96853; and Christine M. Pink, PhD, Joint POW/MIA Accounting Command, 310 Worchester Avenue, Bldg 45, Joint Base Pearl Harbor-Hickam, HI 96853-5530*

After attending this presentation, attendees will see that standardized locations of the adult human femoral midshaft lose cortical thickness differentially with increasing age. Discovery of a uniform pattern of bone loss among this variability may allow for production of age-at-death estimates from unidentified skeletal remains.

This presentation will impact the forensic science community by eliminating a method proposed to utilize multiple types of femoral midshaft cross-sectional data to produce age-at-death estimates from unidentified adult human skeletal remains.

Literature review indicates that once the femur is fully developed, biomechanically adapted, and periosteally adjusted, a net loss of cortical bone begins as the amount of bone deposited on the periosteal surface lessens in comparison to the amount of bone removed from the endosteal surface. Decreasing bone gain on one surface and increasing loss from another changes the size, shape, and strength of the femur throughout adulthood.

To determine whether differential cortical bone loss occurs that can be exploited for production of age-at-death estimates, cortical thickness data was collected from 16 standardized locations (0º (anterior), 22.5º, 45º, 67.5º, 90º (medial), 112.5º, 135º, 157.5º, 180º (posterior), 202.5º, 225º, 247.5º, 270º (lateral), 292.5º, 315º, and 337.5º) around each of 200 adult femoral midshaft cross-sections originally harvested by M.F. Ericksen from George Washington University (GWU) dissecting room cadavers. The sample was composed of 97 males and 103 females largely of European descent, ranging in age from 30 years to 97 years (mean=71 years, standard deviation=12 years).

Results indicate median cortical thickness differs significantly around the femoral midshaft ($X2(15)=609.567$, $p<0.0005$). Specifically, post-hoc pairwise comparisons discovered 71 statistically significant differences between median cortical thicknesses ($p\leq0.05$). From these results, a general pattern emerges where the smallest median cortical thicknesses occur in the anteromedial and posterolateral quadrants of the femoral midshaft, while the anterolateral quadrant possesses all of the largest cortical thicknesses, with the one exception of the posterior 180º location — the linea aspera. Additionally, Pearson and Spearman's rank order correlations found moderately negative statistically significant correlations between age and all cortical thickness locations, with the highest grouped among the anterior, anteromedial, posterior, and posterolateral femoral cortices, and lowest among the medial and lateral femoral cortices. This finding, combined with locally weighted scatterplot smoothing curves of the data, suggests cortical thickness is not lost uniformly with age; rather, the anterior and posterior cortices lose more thickness beginning earlier than the medial and lateral cortices.

An age-predicting linear regression equation utilizing all cortical thickness location data revealed Standard Error of Estimate (SEE) and adjusted R2 values (±10.82 and 0.1957, respectively) comparable to those produced using standard histological methods. Unexpectedly, equations composed from only anterior and posterior cortex data did not perform superiorly. Rather, the best performing equation, Age=89.18+(1.99×135º)+(-2.08×157.5º)+(-3.66×225º), revealed similar SEE and adjusted R2 values (±10.58 and 0.2314, respectively). This equation was tested on an independent sample of 22 femoral midshaft cortices obtained from The Ohio State University Department of Anatomy. Comparison of known ages at death to point age estimates revealed inaccuracies as high as 40 years.

Given these results, previously collected biomechanical and histological data were additionally included to determine what combination of cortical thickness, biomechanical, and histological variables was associated with the lowest SEE and highest adjusted R2 value. The best performing equation, Age=96.10+(-7.49×Ix/Iy)+(-3.67×225º), again produced a high SEE and poor adjusted R2 value (±10.58 and 0.2307, respectively).

Overall, these findings reinforce how femoral midshaft variables primarily reflect mechanical environment regardless of age and suggest there is too much variation in mobility among modern humans for femoral shape, thickness, or remodeling data to be useful indicators of age at death, in any combination.

Age-at-Death, Cortical Thickness, Multiple Linear Regression

A100 Transformation of the Department of Defense's (DoD's) Central Identification Laboratory (CIL): A Historical Review of Its Scientific Personnel and Primary Architects as It Embraces the Tides of Change

MariaTeresa A. Tersigni-Tarrant, PhD, Saint Louis University School of Medicine, Center for Anatomical Science, 1402 S Grand Boulevard, M306, St. Louis, MO 63104; and Denise To, PhD, JPAC-CIL, 310 Worchester Avenue, Bldg 45, Joint Base Pearl Harbor-Hickam, HI 96853*

After attending this presentation, attendees will be provided with a pertinent example of the Academy's 2016 Meeting theme of "Transformation: Embracing Change." The DoD parent organization of the CIL has again reorganized to streamline efforts in its noble mission. After this presentation, attendees will better understand the history of the CIL, its primary architects, and scientific personnel.

This presentation will impact the forensic science community by illustrating how the history of the CIL, with respect to its practitioners and research, has influenced forensic science. This historical review of the CIL may encourage attendees to pay greater attention to changes made by the DoD as to how it chooses to integrate forensic anthropologists to contribute to identification, since this precedent may influence how forensic anthropology is applied in other medicolegal settings worldwide.

The mission of the DoD's Defense POW/MIA Accounting Agency (DPAA) is to search, recover, and identify service personnel still missing from past United States conflicts. The DPAA represents the January 2015 merger of three federal organizations (the Joint POW/MIA Accounting Command (JPAC), Defense POW/Missing Personnel Office, and Life Sciences Equipment Laboratory). The DPAA's Laboratory is the scientific nucleus of the mission, as its anthropologists regularly direct archaeological recovery operations, analyze skeletal material accessioned from these global recoveries and disinterments, and conduct analyses on incident artifacts, all for the goal of positive forensic identification and casualty resolution of our missing service personnel.

The recent merger and transformation of the DoD's accounting effort is nothing new to the Laboratory, as its history includes various transitions and names, although the terms Central Identification Laboratory have been at its core since 1948. This history includes the CIL at Schoffield Barracks in Hawaii, the Central Identification Unit in Japan, laboratory presence in various mortuaries in the Philippines and Vietnam, the CIL-Thai in Thailand, the CILHI in Hawaii, and the JPAC-CIL (at Joint Base, Pearl Harbor-Hickam, Hawaii and Offutt Air Force Base, Nebraska). Most recently, the CIL has become the DPAA-Laboratory. As such, the transformational history of the CIL's participation in the DoD's efforts to identify missing service personnel reflects the history of forensic anthropology itself. The CIL's chief architects and influential scientific staff include Mildred Trotter, T. Dale Stewart, Ellis Kerley, Thomas McKern, and Thomas Holland (among many others). Hundreds of forensic anthropologists (including many Diplomates of the American Board of Forensic Anthropology (ABFA), forensic odontologists, and forensic archaeologists have worked at the CIL. The subsequent global influence of the CIL throughout forensic science is unquestionable.

The CIL has never been autonomous, as it has functioned under the roof of federal organizations, including the United States Army and Navy; however, the CIL's primary obligations have always been to science itself, by practicing and maintaining excellence in the field. While sometimes incongruent priorities and laypeople's good intentions pull the Laboratory in disparate ways, the CIL's commitment to the best practices of forensic anthropology and forensic archaeology is unwavering. Its international accreditation in crime scene processing, forensic biology, and trace evidence analysis has paved the way for other laboratories to do the same. The CIL was integral in responding to the National Defense Authorization Act (NDAA) 2010 National Academy of Sciences' call for improvements in forensic science by co-founding, with the Federal Bureau of Investigation (FBI), the Scientific Working Group in Anthropology (that formulates best practices for forensic anthropologists), which subsequently transformed into the National Institute of Standards and Technology's Department of Justice Organization of Scientific Committees Anthropology Subcommittee. Additionally, its regular activities in many foreign countries have helped shape the global growth of forensic science.

For the DPAA Laboratory to embrace this most recent parental change to its structure, it is therapeutic to acknowledge the historical legacy of its predecessor organizations, as well as acknowledge the contributions that former CIL scientists have made to many subfields in the forensic sciences. This presentation provides a visual representation of the past, so that we may be better prepared to embrace the future. Additionally, given the heavy involvement of federal lawmakers in the transition of the CIL to the DPAA Laboratory and the scrutiny on the forensic sciences in the United States, it is important that practitioners are aware of this transition process and how its ramifications can affect the discipline.

Forensic Anthropology, CIL, Department of Defense

A101 Thirty Years of the Forensic Data Bank and Data Collection Procedures (DCP) 2.0: Continuity and Transformation

Stephen D. Ousley, PhD, Dept of Anthropology/Archaeology, Mercyhurst University, 501 E 38th Street, Erie, PA 16546; Richard Jantz, PhD, University of Tennessee, Dept of Anthropology, Knoxville, TN 37996-0720; Natalie R. Langley, PhD, Lincoln Memorial University, DeBusk College Osteopathic Med, 6965 Cumberland Gap Parkway, Harrogate, TN 37752; Kate Spradley, PhD, Texas State University, Dept of Anthropology, 601 University Drive, San Marcos, TX 78666; and Beatrix Dudzik, PhD, 250 S Stadium Hall, Knoxville, TN 37996*

After attending this presentation, attendees will learn some results of 30 years of data collection and new directions with the Forensic Data Bank (FDB), an important resource for forensic anthropology.

This presentation will impact the forensic science community by highlighting the ongoing growth and evolution of the FDB, assuring its continued relevance to forensic anthropologists.

The FDB was established through a grant from the National Institute of Justice (NIJ) in 1985 to meet the recognized need for updated osteological data. Modern forensic cases could not be analyzed adequately based on data from 19th-century anatomical collections.[1] For 30 years, the FDB has been a source for much of the research in forensic anthropology.[2] Numerous researchers have requested and used data from the FDB. The main goal of the FDB — to update data for estimates of sex and ancestry — has been accomplished, but data collection, like human variation, is an ongoing process. The data have been used to more fully appreciate the secular changes in American groups that have occurred over the past 200 years. Also, reflecting demographic trends, the data have expanded to include a substantial number of Hispanics, the largest ethnic group in the United States, which includes individuals from at least six countries from North, Central, and South America.[3]

Contributions from other forensic anthropologists, which were to sustain the FDB, have waxed and waned, but thanks to the efforts of Richard Jantz, data collection through the University of Tennessee (UT) students has been ongoing and includes new and expanded data sets. For example, when the FDB began, 3D digitizers were unknown in anthropology. Digitizers record 3D landmark coordinates, which can be analyzed using Geometric Morphometric (GM) methods. Additionally, Interlandmark Distances (ILDs) can be calculated from the landmark coordinates. Both GM analyses and ILDs have proven valuable in the statistical classification of human remains. Digitizers have figured prominently in FDB data collection, with nearly all craniometric data collected in the past 15 years using a digitizer. In recent years, more cranial and postcranial data have been collected from Mexico, Guatemala, Germany, South Africa, Japan, Korea, China, and Thailand.

Thanks to the collection of traditional craniometric and postcranial measurements, new methods were developed for statistical classification methods using FORDISC® or other programs and have made it easier to understand morphological variation and sexual dimorphism in American groups.[4] Stature estimation has been greatly simplified, refined, and standardized thanks to FDB data and FORDISC®. The new methods of estimating ancestry, sex, and stature demonstrate the synergistic connection between data and practical applications to help answer forensic questions. The ongoing FDB data collected will reflect upcoming measurement changes and improvements known as DCP 2.0.

One unexpected result was that age indicators, such as Todd and Suchey phases, have not been as informative as hoped. The scores show much higher correlations to known age than any published blind studies, meaning that most observers scored the indicators after a positive identification and knowing the age of the decedent. Hopefully, recording of other age-informative traits, such as traits that can be used in transition analysis, scored before positive identification, will be more valuable in the future.

More recently, other data, such as dental morphology observations, which have been shown to be useful in ancestry estimation, have been added to the FDB.[5] The new kinds of data in the FDB will be incorporated into new statistical software for sex and ancestry estimation and will necessitate new statistical methods to analyze them, such as logistic regression, Bayesian classification, kernel probability density classification, and k-Nearest Neighbor classification.[6] Additionally, the new software will incorporate machine learning classification methods. Additional features such as outlier detection and data transformations will make the software useful for exploratory data analysis and general research, and routines for handling missing data will help make the most of the reference samples.

The data banking concept was recently extended to subadults through the Pediatric Radiology Interactive Atlas (Patricia), which contains more than 45,000 radiographs from individuals up to 20 years old; other researchers have been compiling measurements, radiographs, and Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) scans, and making them available online.[7] Data and methods from publications, Patricia, and other online sources could also be incorporated into the FDB and software. Age estimation for adults and subadults using transition analysis and other methods will also be part of the software package, which will make it much easier to record wide-ranging standardized osteological observations and submit information to the FDB.

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Forensic Data Bank, Statistical Methods, Human Variation

A102 Multidisciplinary Approach of Forensic Science in Historical Study: St. Fortunato of Serracapriola (Italy)

Francesco Sessa, MS, Ospedale Colonnello D'avanzo, Viale Degli Aviatori 1, Foggia 71100, ITALY; Gabriela Perilli, MD, Viale degli Aviatori 1, Ospedale Colonnello D'Avanzo, Foggia 71100, ITALY; Christian Zammit, MD, University of Malta, Dept of Anatomy, Faculty of M, Msida, MALTA; Santina Cantatore, Viale degli Aviatori 1, Foggia 71100, ITALY; Fabrice F. Dedouit, 1 Avenue Du Professeur Jean Poulhes, Toulouse Cedex 9, FRANCE; Giuseppe Guglielmi, PhD, Viale Pinto, Foggia 71100, ITALY; and Cristoforo Pomara, MD, PhD, University of Foggia, Dept Forensic Path, University of Malta, Dept of Anatomy, Faculty of Med & Surg Biomedical Sci, Foggia, Misida, Malta 71100, ITALY*

After attending this presentation, attendees will appreciate St. Fortunato of Serracapriola (Italy) as a historical figure and the role that forensic science can play in bringing scientific evidence to support historical findings.

This presentation will impact the forensic science community by presenting a multidisciplinary approach in forensic science to the remains of a historical figure venerated as a Saint by the Catholic Church.

This presentation discusses the case of St. Fortunato, who lived in Rome in the 3rd century AD. Although there are no historical references about St. Fortunato's martyrdom, his death dates back to the persecution of Christians under Maximinus Thrax, the Roman Emperor from 235 AD. In 1761, St. Fortunato was hailed patron *minus principalis* of Serracapriola, a small town in the area of Foggia (Puglia region), and in 2010 (250 years later), his remains were exhumed by mandate of the clergy for a complete forensic analysis. It is rare that such a forensic multidisciplinary approach is applied to the study of ancient sacred human skeletal remains, as finding data not consistent with St. Fortunato's life might bring into question the believers' faith.

After exhumation, all bones were cleaned and classified according to anatomical topography. A detailed description of bone status was performed and osteological measurements were taken. Multi-Slice Computed Tomography (MSCT) was performed. The elements were placed on the scan table anatomically for easier radiological interpretation; scans were filtered for specific elements in order to determine sex, age, stature, and any pathologies. The radiological data were consistent with a young male, 153cm to 165cm tall, with no signs of pathological or traumatic conditions. Permission was granted for a piece of bone (7g of femur) to be radiocarbon dated. The resulting date indicated an age of approximately 230 AD (95.4%), which is compatible with St. Fortunato's death.

A complete genetic analysis was performed in order to genotype the bony samples. Forensic methodologies were therefore applied in this study, because ancient DNA is often problematic to study given its inherent nature. In ancient skeletal remains, the quantitative and qualitative differences in results can be attributed to environmental factors or to storage conditions. In addition, when dealing with religious relics, one is often limited to investigating a small sample.

Previous protocols were slightly amended in order to improve the DNA quality and minimize the need for the use of expensive equipment and chemicals, yet still ensure a technique compliant with those adhered to by molecular biology laboratories. A complete Short Tandem Repeat (STR) panel was obtained using a low initial concentration of extracted DNA. In accordance with radiological analysis, this data confirmed that the skeletal remains analyzed belonged to a European male rather than one of a different ethnicity.

Although STR profiling is preferred due to its discriminatory power, mitochondrial DNA (mtDNA) analysis is often utilized in these cases. A mtDNA analysis was performed with a Mini-Primer Set (MPS) amplification strategy, following the guidelines described by Melton et al., Budowle et al., and Butler.[1-3] The profile, in terms of differences from the Anderson sequence (i.e., the Cambridge Reference Sequence (CRS)), was compared with databases to determine haplotype frequency. This analysis confirmed that the skeletal remains belong to a European man rather than one of a different ethnicity.

Using multidisciplinary forensic recovery methods, the lines of evidence used toward the identification were: (1) radiological investigations for preliminary information; (2) radiocarbon analysis for dating of skeletal remains; (3) biological profile of the remains (STRs and mtDNA); and, (4) statistical analysis of genetic data.

In conclusion, despite the inability to perform a DNA matching test, this study demonstrates the relevance of a multidisciplinary approach that significantly helped in gathering a variety of information that was consistent with the historical findings about the saint's life. There is a strong scientific concordance between these findings and the époque of the saint's existence, sex, and ethnicity.

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A103 Infant Bone Health: An Evaluation of Quantitative Ultrasound

Miriam E. Soto Martinez, MA, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; Jennifer C. Love, PhD*, OCME, 401 E Street, SW, Washington, DC 20024; and Weilu Han, MPH, University of Texas Health Science Center, 7000 Fannin Street, Houston, TX 77030*

After attending this presentation, attendees will understand the value of Speed Of Sound (SOS) as a measure of infant bone health.

This presentation will impact the forensic science community by evaluating Quantitative Ultrasound (QUS) as an instrument to measure infant bone health. Evaluating pediatric bone health is an important component of a physical exam, especially when unexpected skeletal fractures are found. Research suggests that QUS is a promising tool for assessing infant bone health.

QUS is a technology which measures the speed (m/s) of an ultrasound wave (SOS) as it travels through bone. Studies have shown that physical and material properties that influence bone strength also influence SOS. Bone Mineral Density (BMD) and elastic modulus are positively correlated with SOS, indicating that greater SOS value is related to greater BMD and stronger bone. Cortical thickness, porosity, and anisotropy have also been shown to affect SOS. Furthermore, significant correlations have been reported between bone strength measured by mechanical testing and SOS. As a result of previous research, this study hypothesizes that SOS is associated with factors that affect bone strength, such as chronic illness and prematurity.

In order to test the hypothesis, a prospective study of infant decedents was conducted. During a nine-month period, all infants ranging from 30 weeks post-menstrual age to one year at the time of death that were autopsied by the Harris County Institute of Forensic Sciences and Texas Children's Hospital were included in the study. For each infant, the following measurements and imaging were collected: SOS measurement of the tibial midshaft, circumference of the leg, digital radiographs of the leg and arm, and a histological sample of an anterior rib and iliac crest. Several measurements were collected from the radiographs, including Tibial Midshaft Diameter (TD), total Cortical Thickness (CT), and Medullary Cavity Diameter (MD). Cortical Index (CI), the cortical thickness/tibial midshaft diameter, was calculated. Additionally, the medical histories and autopsy findings were recorded for each decedent.

Analysis of Variance (ANOVA) and linear regression analyses were used to test the relationship between SOS and bone dimensions and medical history (i.e., chronic illness and/or prematurity). The results show no statistically significant differences in SOS measurements between infants positive for traumatic injury or chronic illness and infants with negative histories. A significant relationship was found between SOS and prematurity (p=.011). Simple linear regression analyses indicated that SOS was significantly related to age (p <.001). After birth, SOS decreased with increasing age until ~3 months of age. After ~3 months of age, SOS gradually increased with increasing age until ~9 months of age, at which point it appears to plateau. Removal of the premature infants from the analysis did not appreciably change the relationship between age and SOS. SOS was also significantly related to estimated gestational age (p=.008), birthweight (p=.005), weight (p<.001), weight for age percentile (p=.004), height (p<.001), length for age percentile (p=.018), and leg circumference (p=.002). When age was included as a covariate, there was a significant association between SOS and CI (p<.001) and MD (p<.001). After removal of chronically ill infants from the analyses, the relationships between SOS and TD (p=.017) and CT (p=.043) became significant. After premature infants were removed from analyses, SOS was only significantly related to height (p<.001) and weight (p=.019).

In conclusion, SOS measurements are significantly affected by growth and health-related factors. The significant association between SOS and bone health factors is consistent with previous research and suggests that QUS is an effective tool for evaluating bone strength in infants. Before QUS may be considered a valid tool for evaluating infant bone strength, more research is needed to identify other factors that may significantly affect bone SOS readings in infants.

Quantitative Ultrasound, Infants, Bone Quality

A104 Reliability of Biomechanical Descriptors to Assess Blunt Force Injuries in the Cranium

Ericka N. L'Abbe, PhD, University of Pretoria, 9 Bophelo Road, Pretoria, FL 0001, SOUTH AFRICA; Steven A. Symes, PhD, Mercyhurst University, 501 E 38th, Erie, PA 16546; Michael W. Kenyhercz, PhD, University of Tennessee, 250 S Stadium Hall, Knoxville, TN 37996; Kyra E. Stull, PhD, Idaho State University, Dept of Anthropology, 921 S 8th Avenue, Stop 8005, Pocatello, ID 83209; Gabriele C. Kruger, MSc, 6 Casa Bari, 574 Jacobs Street, Gezina, Pretoria, Gauteng 0084, SOUTH AFRICA; Marie Christine Dussault, PhD, University of Pretoria, Department of Anatomy, Basic Medical Sciences, Pretoria, Gauteng, SOUTH AFRICA; Leandi Liebenberg, MS, 15 Bentwood, Uys Krige Street, Bloemfontein, Free State 9300, SOUTH AFRICA; Erin Chapman, MS, MA, 501 Kensington Avenue, Buffalo, NY 14214; and Jolandie Myburgh, MSc, Univerist of Pretoria, Room 5-17, Basic Medical Sciences Bldg, 9 Bophelo Road, Pretoria, Gauteng 0001, SOUTH AFRICA*

After attending this presentation, attendees will better understand the reliability of features used to describe and interpret blunt force injuries in the cranium.

This presentation will impact the forensic science community by contributing to knowledge on the reliability of descriptions to evaluate bone fractures associated with blunt force injuries.

The predictable response of human bone to destructive forces has been demonstrated in experimental and observational research.[1-4] Bone fracture variability associated with blunt trauma is a combination of intrinsic and extrinsic factors. Biomechanical descriptions are fundamental to evaluating fracture variation and for interpreting the mechanism of traumatic injuries, but the reliability of biomechanical applications to bone trauma interpretation has rarely been tested on real-life forensic cases.

The purpose of this study is to address the reliability of anthropologists to interpret multiple blunt force injuries to the cranium using nine biomechanical descriptions consistently reported in case reports and published in the literature. Among these are included: minimum number of impacts; internal surface radiating fractures; internal surface angled-bevel (failure in compression); external scratch on the bone's surface; chipping of bone; depression or distortion of outer bone contour (failure in external tension); and radiating fractures. Six crania with previously categorized blunt force injuries were randomly selected from the evidentiary archives of cold cases in the Department of Anatomy at the University of Pretoria, South Africa. Six observers, who ranged in bone trauma experience from none to greater than 20 years, were provided with both written definitions and photographs, documenting the above-mentioned variables. Only the two observers had prior knowledge of the cases. All variables were scored following a dichotomous system (present or absent). The scores were summed into a "total trauma score" for each skull and each observer.

Percent agreement was used to evaluate the reliability of each feature among all six observers. A Total Trauma Score (TTS) was also created for each observer. The TTS acted as a component score for all traits for each cranium. An Intra-Class Correlation Coefficient (ICC) was used as an index of inter-rater reliability.

Depression or distortion of bone (plastic deformation) and external scratch on the bone's surface were the most reliable with percent agreements at 98.9%, followed with chipping of bone at 88.9%. The remaining traits ranged between 70% and 78%. The ICC for TTS was 78% (p-value=0.001) and had a 95% confidence interval of 36%-96%. When separated by experience, the observers with the most years of experience showed the most disparity among TTS (ICC=58%) and the observers with the least years of experience had less disparity among TTS (ICC=71%).

Knowledge of basic biomechanics and fracture pattern recognition is key to accurate interpretation of trauma in the human skeleton. Additionally, bone trauma interpretation is a complex, multifactorial process not easily amenable to statistical analysis. Yet, when biomechanical descriptions are evaluated within a dichotomous system, a comparison of bone trauma trait analysis among anthropologists of varying experiences is possible. Overall, the biomechanical descriptions are repeatable among observers of various experiences. Experience (in years) did not seem to positively affect the ICC, but bias may exist as some of the experienced observers had previous experience with the material.

The findings offer support for the use of the biomechanical approach to describe, analyze, and interpret traumatic injuries in bone. Within a biomechanical framework, observations related to blunt trauma interpretation are more likely to be reliable than simply classification methods. Future studies will include descriptions of ballistic injuries and will address the definition/refinement of blunt traits with lower reliability.

Accurate interpretation of fracture characteristics is necessary for communication among scientists, in education, and in reports submitted to a court of law.

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Bone Trauma, Reliability, Biomechanics

A105 Semi-Automated Volumetric Quantification of the Frontal Sinuses: Sexual Dimorphism in a Contemporary Australian Subadult Population

Reanna J. Morris, Queensland University of Technology, George Street, Brisbane, Queensland 4001, AUSTRALIA; Nicolene Lottering, BS, Queensland University of Technology, School of Biomed Sci, Faculty of Health, 2 George Street, Gardens Point, Brisbane, Queensland 4001, AUSTRALIA; Mikaela S. Reynolds, MSc, Level 5 Q Block,, 2 George Street, Gardens Point, Brisbane, Queensland 4001, AUSTRALIA; Laura S. Gregory, PhD, Queensland University of Technology, School of Biomedical Sciences, Gardens Point Campus, Brisbane, Queensland 4001, AUSTRALIA; and Donna M. MacGregor, MSc, Queensland University of Technology, School of Biomedical Sciences, Faculty of Health, Gardens Point Campus, Brisbane, Queensland 4001, AUSTRALIA*

After attending this presentation, attendees will appreciate: (1) the benefits of a semi-automated protocol to conduct volumetric measurements of complex skeletal elements using tissue density-based voxel-processing of computed tomography scans; and, (2) the presence of sexual dimorphism in frontal sinus volume in subadults.

This presentation will impact the forensic science community by advancing understanding of subadult frontal sinus development through the use of 3D reconstructed Multi-Slice Computed Tomography (MSCT) scans. This research will also reinforce the invaluable nature and varied applicability a contemporary virtual skeletal repository offers to both forensic anthropology and osteoarchaeological fields of research.

Due to high probability in the recovery of the frontal bones within fragmented remains, comparison of frontal sinus morphology in antemortem versus postmortem radiographs are routinely conducted for personal identification in forensic casework. While sexual dimorphism in frontal sinus size has been widely reported in adults, there is currently no research signifying the age of onset of sexual dimorphism in morphometric analyses of the frontal sinuses in subadults. Accordingly, this study seeks to establish a temporal profile of frontal sinus development and determine the timing of sexual dimorphism of the frontal sinus volume in a contemporary population of Australian subadults, using a standardized 3D modeling protocol.

A total of 89 cranial Digital Imaging and Communications in Medicine (DICOM) datasets (males: n=45, females: n=44) aged 6 years to 19 years were accessed from the Skeletal Biology and Forensic Anthropology Virtual Osteological Database and imported into an advanced visualization software to generate an isosurface model and segmented masks of the right and left frontal sinuses.[1] A semi-automated method for volumetric calculation was developed to produce a standardized protocol for frontal sinus segmentation based on material discriminatory voxel intensity values. Voxels with intensity values ranging from -1,000 to 150 Hounsfield Units (HU) were selected, corresponding to the tissue density of air, fibrous connective tissue, cellular debris, and mucous that may be contained within the sinus cavity. Frontal chord (Bregma-Nasion) measurements were also conducted to standardize the crania, accounting for individual variability in cephalic size.

Intra- and inter-observer testing was conducted on three sample datasets obtained from randomly selected individuals of varying ages. Intra-observer error demonstrated high precision and consistency between repeated measurements with a mean percent Technical Error of Measurement (%TEM) of 3.15% and Intra-Class Correlation Coefficient (ICC) of 1.00 (Confidence Interval (CI)=0.992-1.00). Similarly, inter-observer reliability demonstrated a high degree of observer agreement despite varying anatomical and radiographic experience, with a mean %TEM of 1.26% and ICC of 0.99 (CI=0.991-1.00). Age and sex effects were analyzed using independent Student t-tests with relevant post-hoc tests.

The preliminary results of this study show a significant expansion of normalized total frontal sinus volume from 6 years to 16 years of age from 7.20±1.75 mm^2 to 99.84±13.16 mm^2 and 12.85±2.91 mm^2 to 65.64±10.49 mm^2 in Queensland males and females, respectively (P <0.01), with the greatest growth velocity occurring between 12 years and 16 years (P ≤0.01) represented by a 2- and 3.6-fold increase in males and females, respectively. Sexual dimorphism of frontal sinus volume was prominent from 12 years of age, with males exhibiting greater absolute and normalized volume than females in the 12 year-13 year, 15 year-16 year, and 18 year-19 year cohorts (P ≤0.05). This is markedly later than that reported in a recent *in silico* study investigating maxillary sinus volume in a Malay population which demonstrated the presence of significant sexual dimorphism after the 6th year.[2]

Interestingly, the data also revealed a 9% bilateral and 3% unilateral absence of the frontal sinuses. Bilateral absence was highest in the 6 year-7 year age cohort, suggesting later pneumatization timings than previously recorded. This presentation highlights the use of voxelization-based *in silico* volumetric approaches to quantify irregular skeletal structures based on their tissue composition. This study provides the first insight into the complex morphological and volumetric changes of the frontal sinuses that occur with age in the cranium of Australian children. Classification accuracies using discriminant function analysis for subadult age estimation of individuals aged 6 years to 25 years of age as a new tool biological profile in contemporary subadults will also be discussed.

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A106 Estimating Age in Juvenile Crania Using Cranial Vault Thickness (CVT)

Kelly R. Kamnikar, BS, Mississippi State University, PO Box AR, Mississippi State, MS 39762; Nicholas P. Herrmann, PhD, Mississippi State University, Cobb Inst Archaeology, Box AR, Dept of Anthro & Mid East Cultures, Mississippi State, MS 39762; Pierre M.M. Guyomarc'h, PhD, International Committee of the Red Cross, Mansour Bldg, Sadat Street, Hamra, Beirut, LEBANON; and Molly K. Zuckerman, PhD, Mississippi State University, Dept of Anthro and Middle Eastern Cultures, Box AR, Mississippi State, MS 39762*

After attending this presentation, attendees will better understand a novel technique of aging juvenile skeletal remains using CVT.

This presentation will impact the forensic science community by offering an alternative technique for aging unknown juvenile skeletal remains.

Age estimation, a component of the biological profile, contributes significantly to the creation of a postmortem profile of an unknown set of human remains, which can aid forensic professionals in linking remains to a missing person's profile. The goal of this study is twofold: (1) to introduce a new juvenile age estimation technique using CVT; and, (2) to compare CVT age estimation in an unknown individual with dental development, a more reliable technique.

Data for this study comes from Computed Tomography (CT) scans (n=74, 37 males and 37 females) of living children in Paris and Bordeaux, France. These scans come from individuals aged newborn to 16 years old and from different ethnic backgrounds. CVT was measured at five craniometric points (nasion, glabella, bregma, lambda, and opisthocranion) that have previously shown correlations between CVT and age, using the Half Maximum Height (HMH) function of the Treatment and Increased Vision for Medical Imaging (TIVMI) software.[1,2] HMH values provide an optimized interface between the tissues (air, soft tissue, bone, etc.) with high accuracy, based on the Hounsfield units of the CT scan. Multivariate Adaptive Regression Splines (MARS) models, using LOcal regrESSion (LOESS) regression, were created to illustrate the relationship between cubed root of known age and CVT in the open-source statistical software, R. A Prediction Interval (PI) was created for each point from each of the models.[3]

Results from this study indicate that CVT data vary in their predictive ability for age by location. CVT data for nasion and glabella do not conform to normality tests, which was further reinforced by visual examination of the Quantile-Quantile (QQ) plots. Models at bregma, lambda, and opisthocranion were normal and indicated that the models fit the data. PIs at bregma varied by 0.4mm, a difference that cannot be reliably used as an age indicator. PI values for lambda range from 0mm to 3mm in thickness and values for opisthocranion range from 0mm to <4mm.

The PIs were then used to assess age in an unknown juvenile cranium, from the Mississippi State Medical Examiner's Office in Jackson, MS. CVT was measured by manually calculating HMH values on a radiograph in ImageJ. These values were compared to PIs created from the models, and low, high, and mean values were produced at the 95% and 85% PI.

At the 95% PIs, predicted values for both lambda (2.214 years to 17.704 years with a mean age of 7.469) and opisthocranion (0.848 years to 22.778 years with a mean age of 6.762) are very large. The mean values remained the same at the 85% PIs, but the ranges narrowed to 3.2175 years to 14.4074 years for lambda and 1.715 years to 17.254 years for opisthocranion. Age estimation using transition analysis of dental development produce an age range of 3.811 years to 7.839 years with a mean age of 5.509 years old.[4]

Comparison of the two methods indicates that the PIs for age estimation using CVT at lambda and opisthocranion are large. The PIs for opisthocranion reach into early adulthood. Results also show that aging by CVT overestimates juvenile age at both points, although the mean at lambda is more overestimated. Aging by CVT is not as accurate as aging by dental development. Aging by CVT could benefit from additional known-age samples in model and PI creation. A larger sample could possibly narrow PIs and make estimates more reliable.

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Age Estimation, Juvenile, Computed Tomography

A107 Correlation Between Body Size and Intracranial Capacity in Korean Youth

Jae gul Suh, MD, Department of Anatomy, Korea University College, Inchonro 73, Sungbuk gu, Seoul, SOUTH KOREA; Yesel Kim, MD, Department of Anatomy, Korea University College, Inchonro 73, Sungbuk gu, Seoul, SOUTH KOREA; Dasom Kim, BA, Department of Anatomy, Korea University College, Inchonro 73, Sungbuk gu, Seoul, SOUTH KOREA; In Sung Park, PhD, Department of Emergency Medicine, Kyungil Univers, 50, Gamasil-gil, Hayang-eup, Gyeongsan, SOUTH KOREA; Nam Joon Lee, MD, PhD, Department of Diagnostic Radiology, Korea Univers, Inchonro 73, Sungbuk gu, Seoul, SOUTH KOREA; and Im Joo Rhyu, PhD, Department of Anatomy, Korea University College of Medicine, Seoul, SOUTH KOREA*

After attending this presentation, attendees will understand how to estimate the intracranial capacity from body size and vise versa.

This presentation will impact the forensic science community by providing estimation equations between body size and intracranial capacity.

Cranial capacity is an important parameter in the fields of evolutionary biology and anthropology and is closely related to brain size. Therefore, cranial shape and capacity are important parameters in evolutionary research, physical anthropology, and forensic sciences.

Four methods are currently available to obtain intracranial volume: (1) a balloon and water filling method; (2) cephalometric measurements and calculations based on estimation equations; (3) the small grain-packing method; and, (4) measurements based on radiological tools, including computed tomography and Magnetic Resonance Imaging (MRI). In this study, 3D modeling of intracranial volume based on MRI data obtained from living subjects was used, not dry skulls of unknown origin.

The subjects were recruited through advertisements at the Korea University web page and local community newspapers. The research purpose and procedures were fully explained to the subjects. History taking (including alcohol consumption) and physical examination were performed by a neurologist of the Korea University Medical Center. MRI was performed on a 1.5-Tesla Magnetom vision after measuring body height and weight. The Digital Imaging and Communications in Medicine (DICOM) format data were imported into the V-work 3.5 program. The MRI signal of the cranial bone was identified under the direction of a radiologist, the intracranial 3D volume model was constructed, and the volume was calculated automatically by the program based on voxel information.

The relationships of body height and weight with intracranial capacity volume differed according to sex. The intracranial capacity volume was correlated with body height in males (R=0.39, p <0.05), but not in females (R=0.24, p >0.05). The correlation coefficient for intracranial capacity and body height markedly increased to R=0.71 (p <0.01) when male and female subjects' data were combined. Conversely, intracranial capacity (volume) correlated with body weight in females (R=0.38, p <0.05), but not in males (R=0.16, p >0.05). The correlation coefficient of body weight and intracranial capacity volume reached R=0.63 (P <0.01) when the data from all subjects were included in the analysis. Further stepwise linear regression analyses revealed that body height is a key variable for intracranial capacity volume, which can be expressed by the following equation: intracranial capacity volume (cm^3)=(11.440 x body height)–420.03, R^2=0.51. The overall correlation coefficient between intracranial volume and brain volume is 0.894 (P <0.01), and linear regression provides the following equation: brain volume (cm^3)=58.3+(0.84 x cranial capacity), R^2=0.795.

In this study, it was shown that intracranial capacity in Korean youth is significantly influenced by body height, and intracranial capacity strongly correlated with whole brain volume. These data will be used to characterize physical anthropological aspects of Koreans and provide a useful tool for forensic application.

Skull Capacity, Body Weight, Body Height

A108 Estimating Body Composition From Stature and Bi-Iliac Breadth in Modern Young Adult United States Populations (NHANES III)

William C. Schaffer, MA, 13229 S 48th Street, Apt 3042, Phoenix, AZ 85044*

After attending this presentation, attendees will have a better grasp of the fidelity to estimate total body mass, fat-free mass, and fat mass from stature and bi-iliac breadth in modern United States individuals and human skeletal remains as well as some of the factors that impede the accuracy of body composition estimates using the stature and bi-iliac breadth technique.

This presentation will impact the forensic science community by supplying an accurate method to estimate body composition for use in forensic anthropology and the potential for the inclusion of body composition estimates in the biological profiles of unidentified human remains for use in medicolegal investigation and human identification.

Often estimates using the stature and bi-iliac breadth technique for total body mass are not presented in biological profiles for use in forensic anthropology because: (1) there is a lack of population- and sex-specific equations for modern individuals; and, (2) many studies using existing techniques to estimate body mass of modern individuals with known mass are inaccurate.[1–7] Recently, greater confidence has been demonstrated in estimating total body mass from stature and bi-iliac breadth using modern young adults (20 years to 39 years of age), but only in individuals with health body fat percentages.[8] Thus, a major caveat when employing the stature and bi-iliac breadth method to estimate total body mass in modern individuals is both age and excess body mass that deviates from healthy norms.[3,8,9]

Body composition was estimated using stature and bi-iliac breadth from 5,555 individuals in six population samples, males and females, who self-identified as non-Hispanic United States White, non-Hispanic United States Black, and Mexican American from the Third National Health and Nutrition Examination Survey (NHANES III) 1988–1994.[10] Fat-free mass was estimated using values extracted from bioelectrical impedance analysis.[11] The population samples were segmented into sub-samples representing groups with healthy body fat percentages as recommended by the American Council on Exercise (ACE) and groups with increments of +5%, +10%, +15%, and +20% body fat above the healthy norm.[12-13] Ordinary least squares regression was conducted for each sub-sample (108 total) using total body mass, fat-free mass, and fat mass as the dependent variables, and stature and bi-iliac breadth independent variables.

The general pattern observed in this study is that when estimating total body mass, with increasing body fat percentages beyond ACE standards, the influence of stature decreases while the influence of bi-iliac breadth increases substantially. When estimating fat-free mass, the estimates are approximately the same in terms of the influence of stature and bi-iliac breadth, even when using various body fat percentages. Noticeably, this pattern is more consistent in males than females for all population affinities. The overall interpretation of this trend is that fat-free mass can be more accurately estimated from stature and bi-iliac breadth irrespective of body fat percentage and that the primary signal in body composition estimation using the stature/bi-iliac breadth method is fat-free mass. Additionally, prediction of fat mass appeared to be the least accurate in individuals with healthy body fat ranges, and increasingly more accurate only when body fat percentage was elevated and fat mass made up a greater proportion of total body mass.

Three ways to estimate total body mass in human skeletal remains using stature and bi-iliac breadth are proposed. First, total body mass can be estimated assuming both that the individual is a young adult (20 years to 39 years of age) and with body fat percentage within a healthy range.[8] Second, fat-free mass can be estimated, then various ranges of body fat percentages can be presented to achieve incremental total body mass estimates. Or third, fat-free mass can be estimated, then modeled expectations of fat mass in healthy individuals that coincide with stature and fat-free mass can be predicted.[14–16]

The results of this study continue to support the stature/bi-iliac breadth method as an accurate technique to estimate not only total body mass, but now also body composition components such as fat-free mass and by indirection fat mass; however, most estimations assume the individual is a young adult and has healthy body fat percentage. The method presented provides a new reliable tool to estimate body composition for inclusion in biological profiles to aid to the process of human identification in forensic anthropology and medicolegal investigation.

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A109 Estimation of Stature From the Foramen Magnum Region in an American Population: A Validation Study

Margarita M. Villarreal, BS, 707 Eucalyptus Street, Ontario, CA 91762*

After attending this presentation, attendees will understand how new univariate and multivariate linear regression formulas were developed for the American White population for the determination of stature from the foramen magnum region.

This presentation will impact the forensic science community by providing a new method to estimate stature using the foramen magnum area for the American White population. While the traditional long bone methods are better for stature estimation, this new technique does provide a reasonable stature estimation range. Thus, if only the cranium is found, a complete biological profile can be assessed.

In forensic cases, complete human remains are not always found, making the ability to estimate stature from bony elements other than long bones important. This validation study focused on Cui's and Zhang's method for the estimation of stature using the foramen magnum region developed for China's Northern and Southern male populations.[1] This study addresses two main research questions: (1) can Cui's and Zhang's regression formulas for Chinese persons of unknown birthplace be used to significantly estimate stature in an American White population; and if not, (2) can new linear regression equations be developed for American White males and females in order to estimate stature within a reasonable margin of error?

Using Cui's and Zhang's 11 parameters from the foramen magnum area and William Bass Donated Skeletal Collection at the University of Tennessee, Knoxville, modern crania from American White females (*n*=137) and American White males (*n*=135) were measured. Age at death varied between females (29 years to 89 years old) and males (26 years to 96 years old). Tests indicate that the regression formulas for Chinese persons of unknown birthplace estimated stature better for females than males, but not significantly. Thus, new simple linear regression formulas representing univariate and multivariate equations were created for American White females and males.

American White females had an interval of Standard Error Of Estimation (SEE) of ±6.32cm to ±6.53cm, with an SEE of ±5.66cm to ±5.95cm for males. Correlation coefficients between stature and measured parameters were different in males and females, except for the Maximum Interior Distance (MxID) between condyles. Blind tests using 40 cranial measurements not used in the creation of the new regression formulas were tested on the new equations for males (*n*=18) and females (*n*=22). When estimated stature is not adjusted for age at death, 77% of total female test cases had their estimated stature fall between 0cm to ±10cm of their recorded stature, and 92% of total test cases fell between the 0cm to ±15cm interval. The same percentages occur when age at death is used to adjust estimated stature in the female test cases; however, this is slightly different for males. When age at death is not used to adjust estimated stature, 72% of total male test cases had their estimated stature fall between 0cm to ±10cm from their recorded stature; when the adjustment is made, a 2% increase is seen with 74% of total cases occurring in the same cm range. In addition, 93% of total male cases had their non-adjusted estimated stature fall between 0cm to ±15cm from their recorded stature; when the adjustment is made, a 2% decrease is seen with 91% of total test cases falling in the same cm range. In addition, inter- and intra-observer tests for reliability conducted showed that only the MxID parameter was poorly reliable when measured with an inter-observer ICC2,1 of 0.254.

In conclusion, this validation study found that the foramen magnum region could not significantly be used to reliably estimate stature; however, in cases where only the cranium is found, it can be used with some accuracy as it is the only cranial method available for the American White population.

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Stature Estimation, Cranium, Foramen Magnum

A110 Examining Four Potential Proxies for Standard Craniometrics: A Statistical Analysis for Significance and Demographic Correlations

Jacob L. Cheramie, 3410 Severn Avenue, Apt 521, Metairie, LA 70002; and Maranda A. Kles, PhD, University of Louisiana at Lafayette, PO Box 40198, Lafayette, LA 70504*

After attending this presentation, attendees will recognize the potential for the development of proxy measurements for standard craniometrics and the need for novel craniometrics. This study presents an evaluation of proxy measurements for Upper Facial Breadth (UFB) and a novel measurement for interorbital distance.

This presentation impacts the forensic science community by providing an alternative method for ascertaining UFB in fragmented remains and presents a potential new cranial measurement. Both of these measurements contribute to the identification of sex and ancestry of human skeletal remains in forensic cases.

Following the contemporary trend in the discipline of forensic anthropology to re-evaluate standard craniometrics and add statistical rigor to the assessment of sex and ancestry, this preliminary research tests three potential proxy measurements for UFB and one potential proxy measurement for interorbital breadth. These proxies would be used for fragmentary remains in which the pristine standard craniometrics are unobtainable.

The three UFB proxies are based on a unilateral measurement from nasion to Frontomalartemporalis (FMT) multiplied by two; one is measured in the same plane ("Planar"), one is measured across the plane ("Cross-planar"), and the last is measured with an instrument ("Apparatus") created for this study. The proxy for interorbital breath was the measurement of distance between the orbits at the height of nasion, as opposed to the standard dacryon-to-dacryon measurement. These measurements were then compared to their standard measurements. Standard craniometrics and the four proxy measurements were collected on 30 individuals, most with known demographics.

Fluctuating asymmetry was assessed and found not to be a factor in the study population. The proxies were then tested for significant difference from their standard counterparts. All measurements were found to be significantly different from the standard, except the "Planar" measurement. The "Planar" proxy and UFB were assessed by discriminant function analysis for their ability to discriminate between the sexes and between the ancestral groups "White," "Black," and "Native American" in both Statistical Package for the Social Sciences (SPSS) and FORDISC®. Analysis shows that "Planar" was as effective as UFB in discriminating sex and ancestry. Based on these results, it appears that the "Planar" measurement is an effective proxy for UFB. Additional testing is needed to further bolster these results.

Testing found the measurement of interorbital breadth at the height of nasion was significantly different than the standard interorbital breadth measurement. Preliminary testing indicates that this novel measurement may, nonetheless, be useful in the assessment of sex and ancestry much like the standard interorbital measurement, but further testing of this finding is needed.

Proxy, Sex, Ancestry

A111 The Effects of Household Corrosive Chemicals on Pig Bones and Human Tissue

Gina E. Baglieri, 160-17 59th Avenue, Flushing, NY 11365*

After attending this presentation, attendees will better understand: (1) how household corrosive chemicals cause decomposition on pig bones and human tissue; and, (2) if altered bones decompose faster than unaltered bones.

This presentation will impact the forensic science community by providing results from a two-part experiment that both attempts to validate previous research as well as adds a new element that has not previously been studied. This presentation will add to our databank of knowledge about the effects corrosive chemicals have on the body.

One of the most important goals in forensic anthropology is to provide information leading to the positive identification of a victim. Positive identification can be challenging because attempts to hide the identity of a victim are frequent. One way to hide a person's identity is the application of household chemicals to a body. These easily obtainable substances may be used to disfigure a body by dissolving the soft tissue and causing it to appear different than it did previously. In recent studies, the effects of corrosive chemicals have been tested on human teeth, bones, hair, and nails. In an experiment by Cope and Dupras, the effects of household corrosive chemicals on human dentition were examined.[1] For the present study, four corrosive chemicals were used: hydrochloric acid, sulfuric acid, phosphoric acid, and sodium hydroxide. The results of this experiment suggest that hydrochloric acid was the most destructive. Similar results are seen in the study by Hartnett et al.[2] The present study examined the effects of household corrosive chemicals on human teeth, hair, nails, and soft tissue. The chemicals included were hydrochloric acid, sulfuric acid, household lye, bleach, and the soft drink Coca-Cola™. The hydrochloric acid was the most destructive agent, fully consuming all tissue in less than 24 hours.

The objective of this research was to study the effects of everyday, household corrosive chemicals on pig bones, human hair, and human nails. The common chemical names and their main ingredients (in parentheses) used in all trials were: Acidic Toilet Bowl Cleaner® (hydrochloric acid), Lime-Away® (sulfamic acid), Septic Tank Cleaner® (hydrogen peroxide), Heavy Duty Stripper and Cleaner® (sodium hydroxide and diethylene glycol monobutyl ether), and Pequa Drain Cleaner® (potassium hydroxide). Water was used as the control. In order to meet the objective, this study consisted of two experiments. The first included recording the effects of the chemicals on the bones, hair, and nails for an extended period of time. The second included recording the effects of the chemicals on altered pig bones. Alterations included burnt, frozen, and chopped bone segments. After an initial pilot study, it was hypothesized that the altered bones would dissolve and change faster than the unaltered bones.

The most destructive chemical tested was the Pequa Drain Cleaner®, which contains potassium hydroxide. It caused the fastest dissolution rate on the burnt bone segment in Experiment #2, causing the bone to become bone residue in only 24 hours. Changes were also seen in both experiments by other chemicals such as the Acidic Toilet Bowl Cleaner®, where the most significant change was also seen in the burnt bone in Experiment #2. A rapid rate of mass loss was recorded from 6.8 grams to 5.9 grams in the first 24 hours. The altered bones dissolved or changed appearance at a faster rate than the unaltered bones, which supports the hypothesis.

Research on the effects of corrosive chemicals can help forensic anthropologists identify cases involving corrosive chemicals and can provide a foundation to direct future research. This research addressed the lack of data on the effects of chemicals on frozen, burned, or cut-up bones, and validated the research by Hartnett et al.[2] It can also be concluded that certain alterations to the bone play a significant role in dissolution rate and overall physical damage to the bone.

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Forensic Anthropology, Corrosive Chemicals, Decomposition

A112 Direct and Indirect Blunt Force Trauma on the Cranium: Any Visible Differences

Kathryn Sloper, BS, Liverpool John Moores University, Byrom Street, Liverpool, Merseyside L3 3AF, UNITED KINGDOM; Constantine Eliopoulos, PhD, Liverpool John Moores Univ, School of Nat Science & Psych, James Parsons Bldg, Byrom Street, Liverpool L3 3AF, UNITED KINGDOM; and Matteo Borrini, PhD, Liverpool John Moores University, RCEAP-School of Natural Science & Psych, Byrom Street, Liverpool L3 3AF, UNITED KINGDOM*

After attending this presentation, attendees will have a better understanding of the possibility of discriminating between the types of cranial fractures resulting from blunt force trauma produced by direct and indirect strikes.

This presentation will impact the forensic science community by providing an empirical experiment model to understand the mechanics of fractures produced as a result of blunt force cranial trauma when the force is applied in both a direct and indirect way.

The majority of studies on the infliction on blunt force trauma to the cranium use mechanical methods (e.g., drop hammers) to produce fractures. These tools require adjustments of both the specimen, to allow for trauma to be inflicted to specific regions, and the amount of force necessary to produce a fracture with each strike.

The goal of this preliminary study was to create an experimental model with participants that inflict blunt force trauma to produce a more realistic fracture pattern.

Twenty adult pig heads (*Sus scrofa domesticus*) were struck with a 16-ounce claw hammer by five right-handed male participants between 20 years and 25 years of age. Specimens were placed in a custom-designed denim bag housed within a holding frame securely suspended by ratchet straps. Each skull was assigned an individual identification code based on both the type of strike (direct or indirect) and the sequence in which trauma was inflicted. For the purpose of this study, a direct strike refers to an over-the-head strike from the right-hand side of the body and an indirect strike refers to an over-the-head strike from the left-hand side of the body. The participants were instructed to strike the frontal region of four pigs' heads using the flat rounded surface of the hammer; two heads were struck using a direct overhead strike and the remaining two heads using an indirect overhead strike. After hot-water maceration, a visual analysis of fractures was performed by comparing with images and descriptions published in previous studies.[1-3]

A total of 16 fractures were observed (average length 42.9mm, width 31.0mm), and the number of lesions produced by indirect strikes (n=9) appear to be larger than direct strikes (n=7). This trend seems to be statistically not significant, but the small sample size must be taken into consideration. A larger sample, for example, may help to discriminate between the types of strike inflicted. Further research involving a quantitative measuring tool (e.g., accelerometer) could also improve the understanding of the dynamics of the force applied to produce trauma.

An additional outcome of this preliminary study is the creation of a flowchart as a supporting tool to identify the fracture type in a more objective and reliable way. The flowchart allows classifying five different types of fractures (linear, superficial depressed, depressed, comminuted, and depressed comminuted) according to the macroscopic characteristics observed on the bone.

Presented here is a pilot study that, even if limited by the small sample size, demonstrates the potential of an empirical experiment and provides an intuitive flowchart that describes the features of the fractures produced as a result of blunt force trauma. Furthermore, this flowchart has the potential to be a more objective method for the description of injuries which could be shared by forensic practitioners.

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Cranial Fracture, Blunt Force Trauma, Biomechanic

A113 A Method of Sex Determination From the Scapula in Modern American Forensics

Melissa K. Kuhn, 5548 Taft Drive, San Jose, CA 95124; Ismail M. Sebetan, MD, PhD*, National University, Forensic Sciences Program, 11255 N Torrey Pines Road, La Jolla, CA 92037-1011; and Amy Zimmer, MS, 1401 Broadway, San Diego, CA 92101*

After attending this presentation, attendees will understand the need for alternative skeletal sexing methodologies and that sexually dimorphic characteristics can be temporally and/or ethnically specific. In addition, attendees will learn that these changing characteristics can also affect discriminant function accuracies over time.

This presentation will impact the forensic science community by offering a new discriminant function method for determining the sex of human skeletal remains from the scapula, thereby allowing construction of a biological profile in which more commonly used skeletal elements (e.g., skull and pelvis) are unavailable.

One of the major goals of the forensic anthropologist is to describe an individual's biological profile, which includes at the minimum sex, stature, age at death, and race.[1] When describing the biological profile, sex determination is completed first because other traits such as stature and age at death can be related to sex.[2] Determining the sex first when building the biological profile is not only important in helping determine other aspects of the profile, but it reduces by half the number of possible unknown individuals needed for comparison.[3] This is especially helpful since the biological profile created by forensic anthropologists is a tool to define as small a group of possible matching individuals as possible from a large group of possible victims and missing persons.[4]

Human skeletal remains are often found fragmented or incomplete. In these situations, postcranial elements such as the scapula are utilized in sex determination and identification methods. A previous study had found that a discriminant function using measurements of the scapula was applicable to a late 19th-early, 20th-century American population. The objectives of this study were to explore if the previous function was still applicable to a more modern American population, if a new, more accurate function could be developed, and if there were any ethnic subgroupings occurring in the United States that could be identified.

This study utilized data from the Forensic Data Bank collected by the University of Tennessee; the previous function was tested and a new discriminant function was developed. The results indicate that both functions can be applied to a modern American population. The previous function achieved an accuracy of 92.4% and the function from this presentation achieved an accuracy of 92.1%. Also, tests were performed to determine if significant ethnic subgroupings were occurring in the study's population data. There were not any significant differences found in the scapular measurements between the White, Hispanic, and Black subgroupings.

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Sex Determination, Scapula, Data Bank

A114 Sex Classification in a Sample of American Whites Using Interlandmark Distances of the Zygomatic Bone and Standard Cranial Measurements

Sarah M. Furnier, 1487 E Hull Road, Hope, MI 48628; and Stephen D. Ousley, PhD, Dept of Anthropology/Archaeology, Mercyhurst University, 501 E 38th Street, Erie, PA 16546*

After attending this presentation, attendees will understand a method of sex classification based on Interlandmark Distances (ILDs) of the zygomatic bone in combination with standard cranial measurements as well as the classification rates achieved in a sample of American Whites.

This presentation will impact the forensic science community by introducing a method of sex classification based on measurements of the zygomatic bone with cross-validated classification rates. This presentation will add to current research by providing an understanding of the utility of ILDs and sexual dimorphism in the zygomatic region of the cranium in sex classification.

Sex classification is a crucial part of estimating the biological profile of an unknown individual. Following the *Daubert* Supreme Court decision, an increased emphasis has been placed on the validation of traditional forensic anthropological techniques, including those for sex estimation, and on the development of statistically sound methods.[1,2] After an extensive literature search, it was found that little has been done with sex classification using metrics of the zygomatic bone. Bizygomatic breadth is generally regarded as one of the best indicators of sex, and previous publications have shown that differences between the sexes do exist in the upper face and zygomatic regions and have obtained high sex classification rates using measurements in these regions.[3,4] Therefore, it is clear that significant sexual dimorphism exists in this region of the cranium. The goal of this study was to determine if ILDs could be used to capture the sexual dimorphism present in the zygomatic and to evaluate whether or not these ILDs, in combination with standard cranial measurements, could accurately classify sex in an American White sample.

ILDs have been shown to be good group discriminators and have been used in the past to successfully estimate ancestry.[5,6] Linear Discriminant Function Analysis (LDFA) has been utilized heavily in metric sex estimation techniques from very early on in the development of metric sex estimation methods.[3,7] In this study, ILDs of the zygomatic bone in combination with standard cranial measurements were analyzed using LDFA in order to examine sexual dimorphism of the zygomatic bone and facial region and to evaluate the sex classification rate in a sample of American Whites from the Terry Collection. FORDISC® 3.1 was used to perform LDFA using stepwise selection to identify the best variables for classification and to provide cross-validated classification rates.[8,9]

Ten measurements — JUB, zygom to zyts, jug to zygoo, zygom to zygoo, fma to zyti, fmt to zygom, jug to zyts, mpl to zygom, fma to zygoo, and ZMB — were stepwise selected using LDFA. An overall classification rate of 82.7% was achieved. Females classified better with 86.5% accuracy in comparison to males at 78.9%. Another LDFA analysis using only unilateral ILDs yielded a classification rate of 78.5%, with females classifying at a rate of 73.7% and males at 82.9%. Five measurements — zygoo to zyti, mpl to zygom, fma to zyti, jug to zygom, and fma to zyts — were stepwise selected in this analysis. In general, the measurement means for males were significantly greater than those for females, except in the case of two ILDs, which were not significantly different between the sexes. Shape analysis resulted in a lower classification but selected variables that reflected width, height, and curvature differences, indicating that there are some shape differences between the sexes.

In conclusion, the results of this study provide evidence that sexual dimorphism does exist in the zygomatic bone. When ILDs of the zygomatic are used, in combination with standard cranial measurements or on their own, this sexual dimorphism can be useful in sex estimation.

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Biological Profile, Zygomatic, Interlandmark Distances

A115 Differential Taphonomy Based on Microenvironment: The Case of Botanical Boy

Kevin M. Lougee, DO, Denver Office of the Medical Examiner, 660 Bannock Street, Denver, CO 80204; James Louis Caruso, MD, OME, 660 Bannock Street, Denver, CO 80204; Meredith A. Lann, MD, Denver OME, 660 Bannock Street, Denver, CO 80204; and Laura A. Regan, PhD, Headquarters United States Air Force Academy, Department of Biology, 2355 Faculty Drive, Ste 2P389, USAFA, CO 80840-6226*

After attending this presentation, attendees will better understand how seemingly minor differences in environment, such as the presence of textiles, can dramatically affect decompositional and taphonomical processes in human remains.

This presentation will impact the forensic science community by providing a unique example of differential taphonomy in a singular burial and offering those involved in human remains recovery and analysis points to consider when approaching potentially similar cases.

The lush area now known as the Denver Botanical Gardens and Cheesman Park, Denver, CO, was originally established in 1859 as the Mount Prospect Hill Cemetery, but due to poor management, disrepair, and alternate ideations for the purpose of the land, interments were largely halted in 1893. Bodies were moved to alternate locations within the city, but graves were poorly marked and burial records virtually non-existent; thus, many decedents remained where they were initially laid to rest.

In October of 2012, human remains were discovered while road construction and irrigation drainage repair were being performed adjacent to the Denver Botanical Gardens. Multiple exhumations were conducted over the following week to include the removal of the remains belonging to a child, estimated at approximately eight years of age at time of death. Coffin hardware and bits of wood were recovered in association with the child. The remains were skeletonized with minor amounts of mummified tissue adherent in the forearm regions. The cranium was present with light-colored hair affixed, combed, and parted. Additionally, the individual was fully clothed in knickers and a jacket (not uncommon dress for young boys of this period) with the suit in exceptional condition. While no headstone was recovered in association with this individual, headstones recovered nearby during this disinterment effort dated from 1878 to 1885. It is not uncommon to find well-preserved skeletal remains owing to the arid climate of the "Mile High City." What is exceptional is to find intact clothing dating back to the 1880s.

The remains were housed in cold storage at the Denver Medical Examiner's Office and were recently examined and fully cataloged so that the unidentified individual may be reinterred locally. The elements in direct contact to the burial environment were quite well preserved; however, those encased within the clothing demonstrated a completely different taphonomy. Large amounts of insect frass were recovered external and internal to the suit. More importantly, the thoracic and appendicular elements contained inside the clothing were delaminated and friable with significant degradation of most cortical surfaces. The slightest movement of these bones led to the osseous material crumbling in place.

This presentation will consider the possible mechanisms behind this differential preservation and what this means to the modern forensic practitioner.

Differential Taphonomy, Microenvironment, Disinterment

A116 Students in the Forensic Laboratory: Fostering Education While Maintaining Quality

Christiane Baigent, MSc, Metropolitan State University Dept Sociology/Anthr, PO Box 173362, Campus Box 28, Denver, CO 80217-3362; and Catherine M. Gaither, PhD, Osa Field Institute, 2066 E Mineral Avenue, Centennial, CO 80122*

After attending this presentation, attendees will have a good understanding of a model for integrating student participation into the forensic laboratory without compromising the probative value of evidence.

This presentation will impact the forensic science community by serving as a tested model for educators seeking to integrate field and laboratory experience into their curriculum, thereby providing a new generation of forensic scientists with invaluable practical experience.

Students and educators at the graduate and undergraduate level colloquially report a lack of internship opportunities in the forensic anthropology laboratory. The fear of compromising the probative value of evidence and irregular and unpredictable caseloads are the most typically cited arguments against internship programs. These fears are not supported by documented courtroom challenges to admissibility and the practice of limiting participation presents a problem for a field that demands experience in order to progress professionally. A thoughtfully designed internship program may provide practical experience without necessitating a predictable caseload and without compromising evidentiary integrity.

The Metropolitan State University of Denver Human Identification Laboratory (MSUD-HIL) offers forensic anthropological search, recovery, and analysis services to medicolegal professionals throughout Colorado. Colorado's complex geography and the changes to biological evidence that occur in these diverse ecozones present challenging educational prospects for search and recovery as well as laboratory analyses not afforded by most classroom settings. In an effort to more effectively process vast outdoor scenes and provide students with the practical laboratory experience necessary for professional development, credited internships are offered to undergraduate students who have demonstrated exceptional academic performance and an interest in pursuing a career in forensic science. The model at MSU Denver utilizes a rigorous Student Quality Assurance (SQA) program in combination with direct supervision of student activities. It offers an alternative whereby budding professionals can gain the practical experience necessary to be successful while maintaining the probative value of evidence and enhancing the investigation.

Introducing an educational platform to scene processing may be beneficial to all involved, but presents a unique set of challenges necessitating foresight, planning, and a strict Quality Assurance (QA) program with the ultimate goal of maximizing the information recovered while maintaining the probative value of evidence. The MSUD-HIL program utilizes a multipronged training and competency testing system specifically designed for student participation, which operates under the laboratory's primary QA protocols. The SQA is hierarchical in nature and devised of a series of benchmarks attained by the successful completion of internal and external training and testing. The level of student participation both in the field and in laboratory analysis is dictated by the certification level achieved. Certification levels are represented by a color-coded system so that qualified (and more importantly, unqualified) students may be readily identified in the field or laboratory, allowing supervisors to easily manage students and rapidly delegate tasks to appropriately qualified individuals.

The simple use of prominently displayed color-coded identification cards has demonstrated the added benefits of self-management and proactive training by student interns. Additionally, students tend to strive to attain higher qualifications with the assistance of more highly certified interns. This affords training experience to more skillful interns while reducing the burden placed on the laboratory director, engenders an ethos of active ongoing education, and demonstrates the importance of QA in the forensic laboratory. SQA is maintained through a series of standardized tests independently assessed by two members of laboratory management at each stage of certification. This ensures that standards are maintained while fostering a multidisciplinary, multi-perspective teaching environment. SQA in the MSUD-HIL required systematic and step-by-step processes, including development of the SQA framework within the primary QA framework, certification manuals, training and commitment among laboratory staff, internal assessment, and integration of SQA programs into the laboratory's annual action plans. Once these controls are in place, students make valuable contributions to any laboratory while reaping the benefits of practical experience. Medicolegal agencies retain the right to deny student participation, but the MSUD-HIL has had few cases where this right has been invoked. Most medicolegal agencies involved with cases where student participation was allowed have praised the students and the laboratory for professional conduct and frequent successful results. Thus, student participation with a rigorous SQA program in place can increase success rates and benefit all agencies involved.

Student Interns, Quality Assurance, Forensic Laboratory

A117 Not All Degree Days are Equal in the Rate of Decomposition: The Effect of Season of Death on the Relationship Between Gross Postmortem Decomposition and Accumulated Degree Days

Lennon N. Bates, MA, Arkansas State Crime Laboratory, 3 Natural Resources Drive, Little Rock, AR 72205; and Daniel J. Wescott, PhD, Texas State University, Dept of Anthropology, 601 University Drive, San Marcos, TX 78666-4684*

After attending this presentation, attendees will better understand the seasonal variation associated with Accumulated Degree Days (ADD) calculations for estimating time since death using gross morphological changes of human remains.

This presentation will impact the forensic science community by demonstrating that seasonal adjustments are needed when estimating time since death from the gross morphological characteristics of human remains in medicolegal death investigations.

The estimation of time since death is an important component of many medicolegal death investigations. Forensic anthropologists commonly calculate the number of ADD necessary to reach the gross morphological changes or stage of decomposition observed on the body.[1] The ADD is then used to estimate the time since death by working backward from the date of discovery until the past date when the calculated degree days are attained; however, seasonal variation in insect activity, humidity, solar radiation, and other factors may affect the ADD necessary to reach different stages of decomposition based on the season of death.[1,2] The purpose of this study was to investigate if there is seasonal variation in the ADD at different stages of decomposition for bodies discovered in an outdoor settings.

Seventy-five individuals donated to the Forensic Anthropology Center at Texas State (FACTS) between 2011 and 2013 were monitored during the decomposition period, and the day each body transitioned from fresh, early decomposition, advanced decomposition, and mummification was recorded.[2,3] The ADD necessary to reach each stage of decomposition was then calculated using local minimum and maximum ambient temperature data. An ADD of zero was recorded if the calculated degrees for a day were negative.[1] Two comparisons were then performed. First, the ADD required to reach each stage of decomposition were compared for bodies placed during traditional seasons: winter (December-February), spring (March-May), summer (June-August), and fall (September-November). Second, the bodies were split into four temperature season categories based on average daily temperature for the month: Temperature Period (TP) 1 — 10°C-15°C (December, January, February); TP2 — 15.5°C-20.5°C (March, April, November); TP3 — 21°C 26°C (May, September, October); and TP4 — 26.5°C-30.5°C (June, July, August). The ADD necessary for each of the following decomposition periods were examined: (1) placement to early decomposition; (2) placement to advanced decomposition; (3) placement to mummification; (4) early to advanced decomposition; and, (5) advanced decomposition to mummification. *T*-tests were used to examine the hypothesis of no seasonal or temperature season variation in ADD required to reach each stage of decomposition.

The results indicate similar decomposition rates for the fall and winter and for the spring and summer. Therefore, the bodies were lumped into two broad seasonal categories: fall/winter and spring/summer. There were statistically significant differences in ADD required for each decomposition period for bodies placed in the fall/winter compared to those placed in the spring/summer. On average, in the fall/winter, 147 ADD were necessary to reach early decomposition, 342 were needed to reach advanced decomposition, and 798 ADD were required from placement to mummification. For the bodies placed in the spring/summer, 76 ADD were needed to reach early decomposition, 209 for advanced, and 512 ADD for mummification. When the bodies were divided based on average monthly temperature, a similar pattern was observed, except for ADD required to reach mummification after placement. There was a negative relationship between average temperature category and ADD required to reach early and advanced decomposition; however, fewer ADD were required to reach mummification in the TP3 (May, September, October) compared to all others. From placement to mummification required 792 ADD in TP1, 737 in TP2, 492 in TP3, and 529 in TP4.

This study demonstrates that it is necessary to control for season of death when using ADD and gross morphological stages of decomposition to estimate season of death. Traditionally, ADD is used in the estimation of time since death rather than calendar days because ADD provides a measure of the thermal energy units available for decomposition and is supposed to take into account temperature differences due to seasonal variation. The results of this study demonstrate that individuals that die during the fall/winter require more ADD than those that die during the spring/summer for all decomposition periods. While temperature is an important factor in postmortem decomposition, seasonal variation in insect activity and other abiotic environmental conditions (e.g., humidity, solar radiation, time below and above thresholds) cause more rapid decomposition that requires less ADD in warmer months compared to colder months in Central Texas.

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A118 A Comparison of Seasonal Decomposition Patterns Between Human and Non-Human Animal Models

Angela M. Dautartas, MA, University of Tennessee, 250 S Stadium Hall, Knoxville, TN 37996; Dawnie W. Steadman, PhD, University of Tennessee, Dept of Anthropology, 250 S Stadium Hall, Knoxville, TN 37996; Amy Z. Mundorff, PhD, University of Tennessee, Dept of Anthropology, 250 S Stadium Hall, Knoxville, TN 37996; Lee Meadows Jantz, PhD, University of Tennessee, Dept of Anthropology, 250 S Stadium Hall, Knoxville, TN 37996-0720; and Giovanna M. Vidoli, PhD, University of Tennessee, Dept of Anthropology, Knoxville, TN 37996*

After attending this presentation, attendees will understand the difference in seasonal patterns of decomposition between humans and two frequently used animal proxies: pigs and rabbits. Attendees will further understand the challenges of applying a commonly used decomposition scoring system to animal remains.

This presentation will impact the forensic science community by discussing whether two species of animal proxies are adequate substitutes for human cadavers for decomposition studies in forensic contexts.

Animal remains are utilized in postmortem interval studies if human subjects are unavailable. It is undetermined if these animal models will yield data that are directly comparable to human patterns, as systematic research comparing decomposition variables between subject species is scarce in the forensic literature. This presentation continues the discussion from the 2015 American Academy of Forensic Sciences (AAFS) Annual Scientific Meeting of a two-year multidisciplinary validation study that compared three cadaver species during three trials that differed by season and microenvironment at the University of Tennessee's Anthropology Research Facility (ARF). The specific purpose of this presentation is to present the results of the analysis of the morphological decomposition rates of all three species across all three trials and seasons; the previous paper detailed only the results of the spring trial.

During each of three trials, five subjects of each species were placed in the same microenvironment at the ARF. Each trial took place during three different seasons (spring, summer, and winter) and in three different microenvironments within the ARF. Decomposition stage was recorded twice daily by applying the Total Body Score (TBS) system of Megyesi et al.[1] Daily photographs were also collected and temperature data was captured hourly.

The TBS scores of all three trials were analyzed using fuzzy clustering. This method was selected because it allows for incorporation and direct comparison of the data from all three seasonal trials. Unlike other clustering methods, the algorithm in fuzzy clustering allows for overlap between group memberships. This is important in this scenario when the question is how much commonality can be found between the patterns of decomposition of the three subject species. The species were compared on the basis of their TBS for specific Accumulated Degree Days (ADD) and also marked by season. If the animal models are a sufficient proxy for human remains, then the animal subjects should show a TBS similar to the humans at the same number of ADD. This would then lead them to be assigned to the same group or cluster.

When all three species were analyzed together, the pigs and humans consistently grouped together in one cluster, while the majority of the rabbits formed their own group. This reflects that the decomposition pattern between pigs and humans is much more comparable than either species is to rabbits. When the rabbits were removed from the analysis to compare only the pigs and the humans, the pigs formed the main cluster, with some of the humans included with the pigs and some humans in a separate group. This reflects the high amount of variability seen in the human specimens as opposed to the consistency across species observed in the pigs.

One of the challenges in this study was applying the TBS system to the animal models. Multiple stages listed in the TBS system rely upon visual cues of decomposition. While indicators such as color changes, skin slippage, and bloat were easily visible on the pigs, the thick fur and small body size of the rabbits often obscured these indicators, making it more difficult to determine stage of decomposition of the rabbits. Use of consistent methods of evaluation is needed in order for research data to be comparable to other studies and must be accurately applied to all specimens.

This project was supported by the National Institute of Justice, Office of Investigative and Forensic Sciences, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the researchers and do not necessarily reflect the views of the Department of Justice.

Reference(s):

1. Megyesi M.S., Haskell N.H., Nawrocki S.P. Using accumulated degree days to estimate the postmortem interval from decomposed human remains. *J Forensic Sci* 2005;50(3):1-9.

Forensic Anthropology, Decomposition, Taphonomy

A119 Around the World in Accumulated Degree Days

Tal Simmons, PhD, Virginia Commonwealth University, Dept of Forensic Science, 1015 Floyd Avenue, Richmond, VA 23284; Colin Moffatt, PhD, UCLAN, School of Forensic & Inv Sci, Preston, Lancashire PR1 2HE, UNITED KINGDOM; Ozgur Bulut, PhD, Hitit University, Facutlty of Arts & Sciences, Department of Anthropology, Corum, TURKEY; Natnipoon Rattanarungruang, BA, 67 Soi Ngamwongwan 21, Bang-khen, Muang Nonthaburi, Nonthaburi, THAILAND; Amanda L. Roe, PhD, College of Saint Mary, 7000 Mercy Road, Omaha, NE 68106; and Donald F. Siwek, PhD, Dept Anatomy and Neurobiology, Program in Forensic Anthropology, 72 E Concord Street, Boston, MA 02118*

After attending this presentation, attendees will better understand how the relationship between Accumulated Degree Days (ADD) and Total Body Score (TBS) can be used to compare and contrast the rate of decomposition across geographic regions around the world.

This presentation will impact the forensic science community by providing results from a controlled experiment conducted simultaneously in five locations in four countries. This presentation will broaden the understanding of how temperature and insects drive the decomposition process, while exploring the effect of local climate and ecosystems.

The vast majority of previously published studies, using either human cadavers or animals, explored specific variables and how these affected the decomposition process; these investigated the effects of individual variables within a single site, with no comparison across different sites. This experiment investigated surface decomposition in five localities in four countries. Research sites included Nakhon Nayok province, Thailand; Ankara, Turkey; Lancashire, England; and Massachusetts and Nebraska in the United States. Average daily temperatures, climatic and geographic conditions, and insect communities varied widely among these sites.

The experiment was initiated in all five localities during the first week of June 2014. Each facility used ten pigs and, with the exception of the Turkish site where pigs were killed by lethal injection, all animals were killed with a captive bolt within one to two hours of being placed at the site. The agreed protocols specified that the pigs should be in the weight range 20kg-40kg in order to minimize body mass differences, as these are known to affect the rate of decomposition.[1] Unfortunately, several sites deviated. Neither Massachusetts nor Nebraska could comply with scavenger proofing and scavenging was observed to some extent at both sites; Nebraska and Thailand clustered at the low end of the specified weight range (21kg and 25kg average weight, respectively), while the other three exceeded the high end (England=43kg; Massachusetts=45kg; Turkey=51kg). As a result of these differences, it is important to note that the data are quite sensitive to the statistical method used and how weight is dealt with within the statistical model.

At all sites, pigs were monitored and observations were recorded approximately every 50 ADD. Observers recorded TBS as well as insect diversity and activity. The pigs were also photographed and scores cross-checked for inter-observer error and internal consistency. Insect taxa were identified by local entomologists working with the observers.

A mixed-effects linear model with TBS as the response variable was used, which took into account the effect of weight as a random variable. The maximum likelihood method was used to produce estimates and their errors. The effects of log10ADD ($F_{1,1086}=18400$, $p<0.0001$) and location ($F_{4,1086}=90.6$, $p<0.0001$) were both significant as was the interaction between log10ADD and location ($F_{4,1086}=224$, $p<0.0001$). The rate of decomposition at different locations was as follows: Nebraska and Massachusetts were similar in rate ($t=1.90$, $df=1087$, $p=0.058$), but different to all others ($t>4.5$, $df=1087$, $p<0.0001$). England was also similar to Massachusetts ($t=1.82$, $df=1087$, $p=0.07$), but different to all others ($t>4.5$, $df=1087$, $p<0.0001$). Turkey and Thailand are also similar ($t=1.61$, $df=1087$, $p=0.11$), but different to all others ($t>13.5$, $df=1087$, $p<0.0001$). The hierarchy in rate was Nebraska > Massachusetts > England >> Thailand > Turkey.

There is much to be considered based on these data, including whether: (1) the rate at which temperature is accrued temperatures (e.g., Massachusetts and Nebraska) affects decomposition rate more than simply the sum of temperatures; (2) the role of fluctuating temperatures affected ADD and insect development; (3) the role of scavenging impacted the unprotected sites; (4) differences in insect communities accounted for varied decomposition rates; and, (5) drug residues in the Turkish animals accounted for slower decomposition.

Reference(s):

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Taphonomy, Accumulated Degree Days, Decomposition

A120 Comparing Decomposition Assessments From Digital Images to In Situ Observations

Gretchen R. Dabbs, PhD, Southern Illinois University, Dept of Anthropology, 1000 Faner Drive, MC 4502, Carbondale, IL 62901; Joan A. Bytheway, PhD, Sam Houston State University, College of Criminal Justice, Box 2296, Huntsville, TX 77341-2296; and Melissa A. Connor, PhD, Colorado Mesa University, 406 Lowell Heiny Hall, 1100 N Avenue, Grand Junction, CO 81501-3122*

After attending this presentation, attendees will learn that the final assessment of the degree of decomposition of a human corpse is not significantly different when the observations of the degree of decomposition are made from digital images of the body taken *in situ* versus when the observations are made directly against the body *in situ*.

This presentation will impact the forensic science community by demonstrating that observation of human decomposition from digital images is a sufficient, and sometimes necessary, method for assessing human decomposition and that the degree of decomposition observed in digital images is not statistically different than that observed on the corpse *in situ*.

In the course of normal events in forensic anthropology, it occasionally becomes necessary to make assessments of the level of human decomposition based on the observation of digital images, instead of the corpse *in situ*. Whether this be for consultation with law enforcement after the deceased has been interred, or for empirical data collection protocols, the question of the degree of agreement between assessments made using these two distinct methods has not yet been addressed in forensic anthropology.

Sixteen participants scored 59 observation packets including digital images using the Total Body Score (TBS) system originally described by Megyesi et al.[1] The participants included both sexes and ranged in education (undergraduate to PhD) and experience (<six months to ten+ years). The packets used 13 human cadavers in different stages of decomposition (Postmortem Interval (PMI) 2 days-186 days) from three outdoor human decomposition research facilities. All observers were recruited for this study from existing human decomposition research facilities and had at least some experience using the TBS method for quantifying decomposition. Observers were provided the scoring tables from Megyesi et al.'s publication and instructed to follow only those descriptions, disregarding any modifications in use by individual facilities and to return categorical scores for each bodily area (head/neck, trunk, and limbs), as well as overall TBS scores. When decomposition fit into more than one category or spanned multiple categories, observers recorded both categories and averaged the contribution to TBS, as instructed by the original publication. Data were collated and the TBS recorded by the project participant was compared to the TBS recorded by the on-site observer at the decomposition research facility the subject was donated to using paired-samples *t*-tests with Bonferroni correction (α=0.003125) (Statistical Package for the Social Sciences (SPSS) v. 22.0).

The average absolute difference in TBS between the on-site and digital image observations ranged from 0.03 to 2.28, with 12 of 16 observers having an average difference in TBS of less than one point. Of the 16 comparisons made, only two cases (12.5% of total sample) demonstrated statistically significant differences between the TBS score recorded by the on-site observer and that recorded by the project participant based on observation of digital images ($p \leq 0.001$). In both cases, the observer of the digital images was an undergraduate student with less than one year of experience assessing decomposition using the Total Body Score method.

Given these findings, it is suggested that observations of human decomposition based on digital images can be substituted for observations based on actual observation of the corpse *in situ* when necessary, as there is generally good agreement between the evaluation of the degree of decomposition using both methods. The one caveat to this statement is that when the observer has little experience (i.e., less than one year), it is best to make this substitution with caution.

This study was conducted with the approval of the Southern Illinois University Human Subjects Review Committee.

Reference(s):

1. Megyesi M.S., Haskell N.H., Nawrocki S.P. Using accumulated degree days to estimate the postmortem interval from decomposed human remains. J Forensic Sci 2005;50(3):1-9.

Forensic Anthropology, Data Validation, Digital Images

A121 Volatile Organic Sulphur Compounds (VOSCs) and Accumulated Degree Days (ADD): Timing the Switch From Anaerobic to Aerobic Putrefaction

Philip E. Houldsworth, MSc, 19 Gynn Avenue, Blackpool, Lancashire FY1 2LD, UNITED KINGDOM; and Tal Simmons, PhD, Virginia Commonwealth University, Dept of Forensic Science, 1015 Floyd Avenue, Richmond, VA 23284*

After attending this presentation, attendees will better understand: (1) the link between VOSCs generated during decomposition and the timing of the switch between anaerobic and aerobic putrefaction; and, (2) how the processes involved in the timing of this switch may increase understanding in the hunt for the elusive Postmortem Interval (PMI).

This presentation will impact the forensic science community by providing data on the VOSCs generated by the putrefactive processes as they progress in individual tissues types. The findings contribute to forensic taphonomic research by improving understanding of the chemical and microbial processes that take place immediately after death and assisting in the goal of improved PMI estimation.

Previously, the research in this area was carried out on whole human cadavers and/or animals. The Volatile Organic Compounds (VOC) were collected by concentrating them from the air above cadavers, which produced large numbers of VOC in very complex patterns.[1,2] The goal of this research was to examine the putrefaction processes in individual tissue types and to analyze the VOC in a controlled laboratory environment in order to simplify the pattern and reduce the number VOC produced within a given time frame. By both reducing and controlling the variables investigated, a better interpretation of the VOC and VOSC production processes and their relationship to Accumulated Degree Days (ADD) is possible.

The research was conducted using tissues (liver, heart muscle, and skeletal muscle) harvested from a freshly killed pig (*Sus scrofa domestica*). Tissues were immediately placed on ice to slow the onset of autolysis. Porcine fecal material was used as a source of enteric bacteria to initiate the putrefactive process. Five grams of tissue were placed into a series of triplicate headspace vials, and 0.5ml of fecal materials was added to the tubes. Along with controls consisting of tissue blanks, air blanks, and fecal material blanks, the sealed vials were placed in a hot air incubator at 37°C for five days to initiate putrefaction. The temperature was monitored and recorded throughout the experiment, so that the amount of VOC produced could be related to the ADD at each sampling point. The VOC in the headspace above the tissue was extracted, separated, and identified by the Headspace/Gas Chromatograph/Mass Spectrograph (HS/GC/MS) at 24-hour intervals for eight days.

The most significant VOC produced during the eight days of data collection were the VOSCs Methyl Mercaptan (MM), Dimethyl Sulphide (DMS), Dimethyl Disulphide (DMDS), and Dimethyl Trisulphide (DMTS). Statistical analysis shows that there is a strong relationship between the production of VOSC and ADD in different tissue types. In liver tissue, the amount of DMDS and DMTS generated was very strongly related to ADD ($p < 0.005$) above 280 ADD. In heart tissue, DMDS was very strongly related to ADD ($p < 0.005$) above 280 ADD, whereas in skeletal muscle, only MM was very strongly related to ADD ($p < 0.005$) above 310 ADD. As DMDS and DMTS are produced by the oxidation of MM and as MM is produced by an anaerobic process, the relationship of these compounds to one another can indicate the presence of anaerobic or aerobic conditions and the switching from one condition to another over time.

In conclusion, the order of decomposition evinced by the results of this study (liver > heart > skeletal muscle) is the same as that described by Gill-King.[3] The switching between anaerobic and aerobic conditions as indicated by the relationship between VOSC and ADD has not been demonstrated previously, and further research is required to establish the determining factors.

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Decomposition, Acumulated Degree Days, Volatile Organic Compounds

A122 An External Validation of the Citrate Content Postmortem Interval (PMI) Method

Michael A. Brown, PhD, College at Brockport, SUNY, Dept of Chemistry and Biochemistry, Brockport, NY 14420; Charles Froome, BS, College at Brockport, SUNY, Dept of Chemistry, Brockport, NY 14103; Shawn Hennessy, College at Brockport, SUNY, Dept of Chemistry, Brockport, NY 14420; Rebecca Gerling, College at Brockport, SUNY, Dept of Chemistry, Brockport, NY 14193; Jeffrey Ellison, BS, College at Brockport, SUNY, Dept of Chemistry, Brockport, NY 14420; and Ann W. Bunch, PhD, SUNY Brockport, Dept of Criminal Justice, 160 Albert Brown Bldg, Brockport, NY 14420*

The goal of this presentation is to provide an update on the external validation of Schwarcz et al.'s method of measuring citrate content of bone to indicate PMI.

This presentation will impact the forensic science community by addressing the potential of Schwarcz et al.'s citrate content of bone method as a predictor of PMI. In addition, its use as an initial sorting tool for ancient versus more recent remains will be discussed.

The PMI of skeletonized remains is a crucial piece of information that can help establish the time dimension in criminal cases. Unfortunately, the accurate and reliable determination of PMI from bone continues to evade forensic investigators despite concerted efforts over past decades to use qualitative and quantitative methods. Qualitative methods have come under greater scrutiny, since the publication of the 2009 National Academy of Sciences Report, Strengthening Forensic Science in the United States – A Path Forward.[1] The numerous quantitative methods (e.g., luminol, radionuclide, carbon-14 bomb spike, and DNA) that have been developed lack the accuracy and/or precision required for reliable PMI estimation.[2-5]

A relatively new PMI method based on the analysis of citrate content of bone was developed by Schwarcz et al.[6] The researchers report that the citrate content of bone decreases with an increase in PMI and that the rate does not depend significantly on storage conditions.[6] Kanz et al. performed an external validation study of this method on cemetery-derived bones with PMIs ranging from ~27 years to 52 years.[7] Their results suggested that the "accuracy of PMI determination was unsatisfactorily low;" nevertheless, the method may show promise for classifying samples as recent or historic.[7] The main objective of this research was to also externally validate the citrate content PMI method and optimize where needed.

More than 50 samples from the University of Tennessee, Knoxville's Forensic Anthropological Research Center and the Onondaga County Medical Examiner's Office were analyzed in this research. The bone samples were prepared using the procedures utilized by Schwarcz et al. with slight modifications to improve method performance. The citrate content (wt%) of each bone sample was determined by an Ultraviolet/Visible spectrometry (UV/Vis) enzyme assay and by High-Performance Liquid Chromatography (HPLC).

Initial studies focused on the assessment of method accuracy, precision, detection limit, and spike recovery. The accuracy for both methods was within ±5 relative error and the precision was less than 2% relative standard deviation. The limit of quantification was ~0.017wt% citrate for both techniques, which is similar to the value reported by Kanz et al. The method reporting limit, which is a more realistic value for PMI determination, was found to be ~0.1wt% citrate for both techniques. A bone sample with a PMI of 173 years was analyzed in order to test the detection limit of the methods and resulted in a citrate value of 0.169 (±0.006) wt % for HPLC and just below the method reporting limit for the UV/Vis assay. Spike recoveries performed for all samples averaged in the range on 95% to 105%. Studies were also performed to establish a baseline citrate content in remains of recently deceased persons (PMI=2 years or less). The baseline was determined to be 1.21 (±0.03) wt% by HPLC and 1.19 (±0.04) wt% by UV/Vis assay. This value is statistically different than the value (2.0 (±0.1) wt%) stated by Schwarcz et al.; however, it is similar to theoretical and experimental values found in the literature.[8-10] Preliminary results from analyzing samples with PMI greater than two years suggest that the theoretical correlation between citrate content of bone and PMI is much weaker than reported by Schwarcz et al., although it is similar to the results of Kanz et al. Despite these findings, this method may still serve as a technique to sort ancient from more recent skeletal cases after further, similar validation studies have been conducted.

This project is funded by a National Institute of Justice Grant.

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Postmortem Interval, Citrate, Skeletal Remains

A123 Differentiating Between Sharp Force Trauma (SFT) Defects and Insect Invasion of Skin of Human Cadavers Throughout the Decomposition Process

Joan A. Bytheway, PhD, Sam Houston State University, College of Criminal Justice, Box 2296, Huntsville, TX 77341-2296; Kevin R. Derr, 28601 Shawnee Court, 192 Waterwood, Huntsville, TX 77320; Zachary Lueck, BS, Sam Houston State University, 10700 Vista Heights Boulevard, Fort Worth, TX 76108; Lyndi S. Turner, BS, Sam Houston State University, 1235 Josey Street, Apt 171, Huntsville, TX 77340; Kandace D. Schakelford, BA, Sam Houston State University, 1742 CR 233, Tyler, TX 75705; Erica N. Fisher, BS, Sam Houston State University, 9318 Floral Crest Drive, Houston, TX 77083; and Luis Dominguez, BS, Sam Houston State University, Box 2296, Huntsville, TX 77341*

After attending this presentation, attendees will understand that SFT skin defects, induced by a variety of sharp instruments, can be recognizable and distinguished from insect invasion defects throughout the human decomposition process into the late phases of the advanced stage. Issues that affect the margins of SFT will also be addressed.

This presentation will impact the forensic science community by providing results of SFT to skin from a controlled but natural environment (simulating how bodies would appear if dumped outdoors and eventually found) study in which no current human taphonomic research exists. This presentation will acknowledge the need for collaboration of human decomposition studies with trauma studies on both hard and soft tissue. This study will also broaden the forensic taphonomy research arena.

SFT in bone and cartilage is well documented in forensic literature and recent literature documents SFT evidence on hair and fabric; however, there is no literature on the effects of taphonomy on SFT of the skin and whether it is identifiable and distinguishable from insect activity throughout the decomposition process.[1-11] In most cases, SFT is identifiable by the pathologist on bodies in the fresh stage of decomposition and observed with less certainty when the body is in an advanced stage of decomposition. It is not known whether particular sharp instruments that produce a certain pattern on the body in the fresh stage of decomposition will maintain that pattern in the late stage of decomposition. In addition, do sharp instruments produce a particular pattern when used in any area of the body and is the pattern visible throughout decomposition? If the patterns are recognizable, can they be distinguished from insect activity?

This study was conducted at the Southeast Texas Applied Forensic Science (STAFS) facility at Sam Houston State University. The climate of southeast Texas is humid and subtropical with a latitude of 30°N, resulting in high heat indices that produce early desiccation in cadaver tissue.[12]

SFT defects of the human skin, how those changed throughout the course of decomposition, and how they were distinguished from insect defects was examined. Eight sharp instruments were used to induce SFT on four human subjects at eight areas of the body: the right and left sides of the neck, right and left abdomen, right and left thigh, and the antero-medial area of the right and left lower leg. Each subject was in a fresh stage of decomposition, unclothed, and in a supine position at the time of the SFT induction. Subjects were protected from scavenging by wire cages. Bodies were accessible to the outdoor environment, including insects. Inflictions at the neck were at an angled, downward motion while those at the abdomen, thigh, and lower leg were downward motions. Instruments used included a Ryobi® reciprocating saw, a Marshalltown® trowel, an Estwing® axe, a Condor® Crocodilian machete, an HDX® clawed hammer, a Dexter Russell® knife, an Ace® shovel, and an HDX® screwdriver.

Length and width measurements were taken of each wound in the fresh stage (Day 1) of decomposition and again 27 days later in the advanced stage. As expected, SFT wounds increased in length and width as decomposition progressed. In some cases, SFT defect edges near the ground tended to mix with decomposition fluid, resulting in a "melted" appearance of the skin, and SFT margins were no longer clearly recognizable for measuring. For SFT measurements that could be taken at both the fresh and advanced stages for each instrument, percentage length increase ranged from 2% to 83%, with the clawed hammer having the largest average percentage length increase (57%). Percentage width increase ranged from 14% to 88%, with the axe having the largest average percentage width increase (76%). All SFT wounds were still evident into the advanced stage of decomposition and were distinct from insect activity defects. Insect defects were predominantly circular in appearance and measured 1mm to 4mm in diameter. Insect defects were located adjacent to SFT defects and consisted of multiple small circular holes clustered together. The holes were also found in areas with thinner skin tissue, such as the shin. The groin areas of all four subjects (where no SFT was created) showed large openings with indistinguishable skin margins dissimilar to SFT openings. The natural orifices of the groin enabled insects to more easily invade and create skin defects that were not observed on other areas of the body.

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Human Decomposition, Sharp Force Trauma, Taphonomy

A124 The "Science of Science": Examining Bias in Forensic Anthropology

Alexandra R. Klales, PhD, Mercyhurst University, Dept of Applied Forensic Sciences, 501 E 38th Street, Erie, PA 16546; and Kate M. Lesciotto, JD, MS, 227 Oakwood Avenue, State College, PA 16803*

After attending this presentation, attendees will understand one form of bias that impacts the interpretation of human remains in forensic anthropological contexts and will better understand how bias impacts the decision-making process. This presentation will further attendees' abilities to join the conversation on how best to incorporate objectivity into the application of forensic anthropological methods.

This presentation will impact the forensic science community by detailing an area of bias that has not yet been studied within forensic anthropology and by engendering a discussion of bias, which is needed to understand how to best move forward with hypothesis-driven research, specifically for generating the biological profile of unidentified human remains.

Since *Daubert*, there has been a push within the anthropology discipline to rely more heavily on objective methods.[1] Recently, the subject of bias when estimating biological profile parameters through non-metric methods has begun to be addressed in relation to objectivity.[2,3] Bias can be introduced into forensic investigations in many ways. The role of forensic anthropologists and scientists is to remain objective; however, recent studies have shown that cognitive bias does impact the interpretation of human remains, as well as the final conclusions drawn, when additional contextual information is provided.[2,3]

The observers' conclusion when assessing biological profile parameters can often "result from expectations about the results of an observation, and such expectations often come either from explicit messages or from subtle cues about the thing to be observed."[4] The goal of this research is to examine the latter, known as the gestalt — that is, the overall impression of the remains when examining individual non-metric traits. To accomplish this, seven experienced observers blindly scored Phenice's three non-metric traits (ventral arc, subpubic contour, and medial aspect of the ischio-pubic ramus) from one (female) to five (male) based on the descriptions and figures from the Klales et al. method for sex estimation.[5,6] To limit the potential influence of contextual bias, participants were informed that they were participating in an observer error study, rather than a bias study. Each trait was scored on a separate day. The 15 innominates were randomly arranged with all identifying markers obscured. Only the specific trait being scored was visible; the remainder of the bone was covered to prevent observation of other traits or robusticity of the bone from influencing the scoring process. On the final day, observers were asked to provide an overall impression of sex and score each trait again, but this time they were allowed to examine the entirety of the bone and all traits simultaneously.

The results indicate a strong confirmation bias, whereby "if one has expectations about an event, or hypothesis (i.e., sex estimation)…one tends to draw selectively from the available evidence and focus on those items that confirm the working hypothesis."[4] When the observer was able to view the entire innominate, including all traits and general size/robusticity, every single observer (100%) feminized or masculinized individual trait scores according to their sex assessment. For example, if the observer originally scored the subpubic contour as 2 (slight concavity), they tended to feminize the score to 1 (well-developed concavity) when they viewed the other traits and estimated the innominate to be female. In the original Phenice article, presence of all three traits was considered to be the female form.[5] Therefore, it is not necessarily surprising that the female innominates were feminized to a greater degree, especially for the subpubic concavity (100% of specimens were feminized by at least one score by observers). The ventral arc was also heavily feminized (83%), while the medial aspect was more consistent: only 33% of females were feminized and only 44% of males were masculinized. Previous research has shown high observer agreement for each of these traits, which further suggests that bias is more likely the cause of within-individual scoring discrepancies.[6]

Bias from contextual information has been previously identified and, with this research, bias from the gestalt has also been confirmed.[2,3] Research and methodology in forensic anthropology must remain hypothesis-driven and objective; the next step in the discipline is to begin a discussion of how best to go about accomplishing this task, given the biases known to be present so that we may better understand the science of our science.

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Bias, Non-Metric Traits, Biological Profile

A125 A Reanalysis of Korean War Anthropological Records to Support the Resolution of Cold Cases

Emily K. Wilson, Defense POW/MIA Accounting Agency, 310 Worchester Avenue, Joint Base Pearl Harbor-Hickam, HI 96815*

After attending this presentation, attendees will be informed concerning the accuracy of 1950s Korean War identification data used to develop certain reference methods.

This presentation will impact the forensic science community by expanding awareness of the complications affecting age at death and stature estimation using 1950s reference methods.

The Defense POW/MIA Accounting Agency Central Identification Laboratory (CIL) has developed a disinterment project to prioritize exhumations of unidentified remains from the Korean War for laboratory analysis and identification. These cases were previously processed at the Central Identification Unit (CIU) in Kokura, Japan, and were buried as unknown. Since 1999, the CIL has disinterred 94 sets of Korean War remains and identified 55 individuals. This process has made available for comparison from each of these 55 cases: (1) the original CIU anthropological assessments; (2) blind CIL assessments made for those same remains; and, (3) the reported antemortem information from each individual identified at the CIL. Zinni presented preliminary comparisons for a portion of these cases.[1] This study presents a comparison of the biological estimations for age at death, stature, and ancestry at both the CIU and the CIL with the reported antemortem information. The purpose of this study is to find patterns of errors that are useful in refining disinterment research (in which more accurate biological profiles are extrapolated from CIU notes prior to exhumation) and in supporting current identification efforts.

The CIU age ranges captured the reported age at death in 58.5% of cases and did not in 41.5% of cases. When the age was overestimated, the reported age was an average of 1.6 years younger than the lower limit of the CIU range (min. 0.25 years; max. 4 years). When the age was underestimated, the reported age was an average of 2.1 years older than the upper limit (min. 0.5 years; max. 4.75 years). CIL age ranges captured the reported age at death in 90.6% (48/53) of cases and did not in 9.4% (5/53) of cases. All five cases for which the CIL range did not capture the reported age at death resulted from underestimation. All five underestimations at the CIL were also underestimations at the CIU. In three of those five underestimations, CIL analysts had exclusively used McKern and Stewart (a method developed from the 1950s Korean War identifications) as a reference.[2]

Reported stature was captured by modified CIU age ranges in 79.2% (42/53) of cases and was not captured in 20.8% (11/23) of cases. When the stature was not captured, 54.5% (6/11) were overestimated and 45.5% (5/11) were underestimated. When the stature was overestimated, the reported stature was an average of 0.9 inches under the CIU estimate (min. 0.1 inches; max. 2.5 inches). When the stature was underestimated, the reported stature was an average of 0.8 inches over the CIU estimate (min. 0.25 inches; max. 1.5 inches). The CIL captured the reported stature in 88.7% (47/53) of cases. When the stature was not captured, 50% (3/6) were overestimated and 50% (3/6) were underestimated. All six cases in which the CIL estimation did not capture the reported stature were also cases in which the CIU did not capture the reported stature, and each in the same direction (over- or underestimated). Trotter and Gleser formed the basis of both the CIU and CIL estimations.[3]

The CIU captured the reported race in 96.2% (51/53) of cases and did not in 3.8% (2/53) of cases. The CIL captured the reported race in 100% (53/53) of cases. Fewer errors are made today at the CIL, but they are the same types of errors. Understanding these systematic errors in 1950s CIU-developed reference data is useful for refining disinterment research and supporting current identification efforts.

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Cold Case, Age-At-Death, Stature

A126 The Status of Unidentified Decedent Cold Cases at the Harris County Institute of Forensic Sciences (HCIFS) From 1957 to 2015

Cate E. Bird, PhD, Pima County Office of the Medical Examiner, 2825 E District Street, Tucson, AZ 85714; Sharon M. Derrick, PhD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; Deborrah C. Pinto, PhD, Harris County Institute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054; Jason M. Wiersema, PhD, Harris County Institute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054; and Jennifer C. Love, PhD, OCME, 401 E Street, SW, Washington, DC 20024*

After attending this presentation, attendees will have a better understanding of how forensic anthropologists have significantly contributed to identifying unknown decedents at the HCIFS.

This presentation will impact the forensic science community by reviewing overall patterns of unidentified cold cases, highlighting the challenges of medicolegal identifications, and summarizing the role forensic anthropologists have played in successfully identifying decedents in the medical examiner setting.

The Forensic Anthropology Division (FAD) at HCIFS has actively pursued the identification of unknown decedents in the greater Houston area since 2006. Currently, the FAD maintains records on approximately 340 provenienced unidentified human remains dating from 1957 to 2015. The majority of the decedents are from the 1980s (35%) and the 1990s (29%), with the number of unidentified cases decreasing steadily to the present. Approximately 78% of the decedents are male and 21% are female, with Whites (37%) and Hispanics (29%) being the most common ancestral groups represented. The manner of death in the majority of the cold cases is either undetermined (43%) or homicide (32%). While 40% of the unidentified decedents have fingerprints available, only 27% of the cases have faces appropriate to disseminate to the public. One of the primary problems with identification of unknown decedents is body condition at discovery. More than two-thirds of the HCIFS cold case remains were decomposing or skeletal at autopsy, which hinders identification because many individualizing characteristics (e.g., facial recognition, fingerprints, etc.) are lost when soft tissue features disappear. A further complication of identification efforts is that even when decedents were not decomposing, traumatic injuries often precluded the possibility of visual identification. In order to increase the likelihood of identifications, the FAD publishes other recognizable attributes of decedents, such as tattoos, personal effects, jewelry, clothing, distinctive fingernails, or dental restorations.

Forensic anthropologists have played a key role in recognizing, organizing, and publicizing unidentified decedent cold cases at HCIFS. Primary responsibilities undertaken by the FAD include reviewing all available records, resubmitting fingerprints to appropriate databases, updating decedent profiles (e.g., National Missing and Unidentified Persons System (NamUs), National Crime Information Center (NCIC), and National Center for Missing and Exploited Children (NCMEC)), performing anthropological analyses, and collecting DNA for analysis and upload to Combined DNA Index System (CODIS). Over the past 10 years, the FAD has received two National Institute of Justice (NIJ) grants, funded two postdoctoral forensic anthropology Fellows, and exhumed the remains of 46 unidentified decedents for the purposes of identification. Due to the concerted efforts of forensic anthropologists at HCIFS, approximately 54% of the unidentified decedent cold cases have DNA submitted or uploaded to CODIS, and all available fingerprint cards have been digitized and resubmitted to appropriate agencies within the past four years. Also, in the last decade, 84 decedents whose cases date back to 1971 have been identified, primarily through fingerprint resubmissions (51%) or DNA matches (36%).

This presentation highlights the demographic trends among the unidentified cold case decedents from Harris County and surrounding areas during the last 50 years. It also illustrates challenges encountered during this endeavor and the significant role forensic anthropologists can play in organizing and resolving unidentified decedent cases in a medicolegal setting.

Unidentified Decedents, Personal Identification, Cold Cases

A127 The ANSI-ASQ National Accreditation Board (ANAB) Accreditation of the Harris County Institute of Forensic Sciences' Forensic Anthropology Division

Christian Crowder, PhD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; Michal L. Pierce, MS, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; and Luis A. Sanchez, MD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will: (1) understand the choices available for forensic agency accreditation and the differences between International Organization for Standardization (ISO) 17025 and ISO 17020; and, (2) learn the challenges faced by professionals practicing forensic anthropology when striving to have their facilities, procedures, and casework meet ISO 17020 standards.

This presentation will impact the forensic science community by discussing the importance of incorporating quality assurance programs and personnel into departmental operations and introducing forensic anthropology laboratory accreditation under the ISO/International Electrotechnical Commission (IEC) 17020 standard.

Following the publication of the 2009 National Academy of Sciences Report, *Strengthening Forensic Science in the United States – A Path Forward*, establishing quality assurance programs and achieving accreditation has become a priority for forensic anthropology practitioners. Until recently, there has been a lack of interest for accreditation boards to develop a program that recognizes forensic anthropology as a named accreditation program. The avenue for accreditation for medicolegal operations is under the National Association of Medical Examiners' (NAME) Standards. While forensic anthropology is mentioned in these standards, it does not specifically provide standards for the practice. The Defense POW/MIA Accounting Agency (DPAA), formally known as the Joint POW/MIA Accounting Command-Central Identification Laboratory (JPAC-CIL), developed a quality assurance program that ultimately led to the laboratory's accreditation by the American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB) in 2003, making them the first credentialed forensic skeletal identification laboratory. In 2008, the laboratory was re-accredited under the ASCLD/LAB-*International* Program in Crime Scene and Trace Evidence using the ISO 17025 Standard. It has been questioned if placing the discipline of Anthropology under a trace laboratory rubric is the most appropriate fit; however, until recently, there were no other options available.

There are currently two ISO standards being used by accrediting bodies to accredit forensic science agencies: (1) ISO/IEC 17025 General requirements for the competence of testing and calibration laboratories; and, (2) ISO/IEC 17020 Conformity assessment-requirements for the operation of various types of bodies performing inspection.

While ISO/IEC 17025 has been widely accepted by crime laboratories for more than a decade, ISO/IEC 17020 has recently gained popularity with crime scene investigation units. The difference between the two standards, and therefore the focus of the respective accreditation programs, is mainly that one addresses analytical instrumentation and data more heavily than the other. ISO/IEC 17025 is geared toward laboratories whose experts report results based on data generated by analytical equipment, while ISO/IEC 17020 recognizes an expert's professional judgment as the means for determining function and acceptability. The latter is more suitable for anthropological examinations.

In 2013, The Harris County Institute of Forensic Sciences (HCIFS) identified a path toward achieving accreditation for forensic anthropology. This path involved building a quality assurance program that followed the American National Standards Institute-American Society of Quality (ANSI-ASQ) ANAB ISO/IEC 17020 Forensic Inspection Agency Accreditation program. Although the ANAB program recognizes a forensic anthropologist as one who reaches conclusions based on functional testing and professional experience, the program also calls for standardization of the work process and examination report. A challenge in building this program internally was finding a way to introduce new training, validation, and record-keeping requirements to experienced staff, who have been using standard methods for several years, without diminishing their sense of professionalism.

In 2015, the HCIFS became the first credentialed forensic anthropology laboratory under this program. Successfully attaining accreditation was a direct result from the Forensic Anthropology Division working closely with the agency's Quality Management Division on a daily basis to build, implement, and maintain the quality assurance program. Using separate quality assurance personnel who do not perform casework and are knowledgeable in basic accreditation requirements is ideal when working toward accreditation. It is recommended that other agencies assign quality assurance staff to assist with implementing and monitoring new quality programs in order to ensure that the proper checks and balances are in place.

Considering the growing momentum of the recently formed Organization of Scientific Area Committees (OSAC) and the continued integration of anthropology in medical examiner/coroner offices, the importance of quality assurance for the anthropology program and surety measures directly related to casework should be emphasized in any agency that utilizes this type of service. The milestone reached by the HCIFS Forensic Anthropology Division will hopefully pave the way for other practitioners to obtain accreditation for their services.

Quality Assurance, Accreditation, Forensic Anthropology

A128 Differential Raccoon Scavenging Among Pig, Rabbit, and Human Subjects

Dawnie W. Steadman, PhD, University of Tennessee, Dept of Anthropology, 250 S Stadium Hall, Knoxville, TN 37996; Angela M. Dautartas, MA, University of Tennessee, 250 S Stadium Hall, Knoxville, TN 37996; Amy Z. Mundorff, PhD, University of Tennessee, Dept of Anthropology, 250 S Stadium Hall, Knoxville, TN 37996; Giovanna M. Vidoli, PhD, University of Tennessee, Dept of Anthropology, Knoxville, TN 37996; and Lee Meadows Jantz, PhD, University of Tennessee, Dept of Anthropology, 250 S Stadium Hall, Knoxville, TN 37996-0720*

After attending this presentation, attendees will understand the differences in postmortem scavenger activity between human subjects and two common animal proxies for human decomposition — pigs and rabbits. Attendees will further understand whether raccoon scavenging of human remains is accurately reflected in animal models of human decomposition.

This presentation will impact the forensic science community by demonstrating whether two species of animal models are sufficient substitutes for scavenger and decomposition studies of human cadavers in forensic contexts.

Animal remains are often utilized in forensic research of the postmortem interval when human subjects are unavailable; however, systematic research that directly compares decomposition variables between subject species is lacking in the forensic literature. As a result, no basis exists to determine whether studies based on animal remains are relevant to situations involving questions of the postmortem interval of human forensic cases. A two-year project at the Anthropology Research Facility (ARF) at the University of Tennessee compared three cadaver species — pigs, rabbits, and humans — across three trials that differ by microenvironment and season. Data collected included insect species and activity, scavenger activity, and temperature and morphological changes in the bodies. This presentation focuses on scavenging across all three trials and specifically examines whether raccoons prefer one species over others and the seasonality of scavenger activity.

The project consisted of three trials in which five subjects of each species — pig, rabbit, and human — were placed across three seasons (spring, summer and winter) at the ARF. All fifteen rabbits were placed in cages to deter scavenging, but an additional rabbit was placed without a cage in Trial 1 to compare possible differences with unencumbered scavenger activity. Game cameras were placed in the study area to capture images of scavengers, document the process of scavenging, and identify which subject species were scavenged. Additional documentation of scavenging activity included daily photographs and notations of scavenging on the subjects, number of scavenger species and number of individual scavengers (when possible), and photographs of animal tracks on and around the subjects.

A total of four scavenger species were documented at the study areas, but their activities varied by season and by subject species. Raccoons were the most commonly observed scavenger, followed by birds (including robins, doves, and cardinals), opossum, and skunk. The birds fed upon the maggots, not subject tissues, while the other species did consume tissues. Raccoons are responsible for the majority of the scavenging. While the human subjects were scavenged in all three trials, the pigs and rabbits were only scavenged in the winter (Trial 3). Rabbit scavenging was limited to removal of fur from only two individuals in Trial 3 and there was no consumption, even though the raccoons could access the rabbits through the cages. All five human subjects were scavenged in Trial 3, but three of the humans were preferred by the raccoons who completed scavenging on these individuals prior to moving to the other two humans or to the pigs and rabbits. Only three of the five pigs were scavenged in Trial 3. The anatomical pattern of scavenging also varied between pigs and humans. Raccoon scavenging of humans typically began on the limbs and could include the head and thorax, while scavenging activity on pigs was limited to the head, neck, back, and abdomen. This reflects the anatomical differences between pigs and humans in terms of muscle (meat) distribution.

Seasonality is a key factor in scavenging for pigs and rabbits, but less so for humans. Although scavengers had access to the same species in the summer and fall, scavenging was most extensive in the winter. Allowed a choice, the raccoons preferred human remains and in some instances even showed preference for one cadaver over another. Thus, variation in scavenging intensity and pattern observed on the human subjects is not captured by the non-human study subjects.

This project was supported by the National Institute of Justice, Office of Investigative and Forensic Sciences, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the researchers and do not necessarily reflect the views of the Department of Justice.

Forensic Anthropology, Scavenging, Taphonomy

A129 Analysis of Taphonomic Changes to Juvenile Pig Bone Exposed to a Marine Environment Using Non-Destructive Raman Spectroscopy

Jennifer L. McDowell, MSc, Sir John Walsh Research Centre, 71 Frederick Street, Dunedin 9016, NEW ZEALAND; Lynne S. Bell, PhD, Simon Fraser University, Dept of Criminology, 8888 University Drive, Burnaby, BC V5A 1S6, CANADA; and Keith C. Gordon, University of Otago, Dept of Chemistry, Union Place, Dunedin 9016, NEW ZEALAND*

After attending this presentation, attendees will understand how a shallow and inter-tidal marine environment affects juvenile bone preservation and will learn the benefits of using Fourier Transform (FT) -Raman spectroscopy to enhance bone analysis, without the need for time-consuming preparation or destruction of samples.

This presentation will impact the forensic science community by adding to the limited research currently available on time-dependent taphonomic changes to juvenile bone (pig) in a marine environment.

The ability to determine immersion time from bone condition is a critical forensic tool, but marine studies have lagged behind terrestrial ones and, in both cases, juvenile bones are far less studied than adult bones.

As forensic research seeks increasingly to exploit the information contained in bone, age-specific knowledge of taphonomic alteration in a marine environment is crucial. Alterations can occur as soon as the early postmortem period, so it is important to be able to distinguish and understand differential bone preservation. The objective of this study was to explore how the chemical composition of juvenile bone alters over time, using FT-Raman spectroscopy. Immersion time and level of submersion (full or partial) were the measured variables. Pig bone was used in this study as a proxy for human subadults, as both pig and juvenile human bone exhibit the presence of Haversian and plexiform bone. This is not a perfect model, but it is useful in examining the effect water has on bony substrate in lieu of the availability of human samples.

Piglet carcasses (*n*=40) were placed in the inter-tidal or sub-tidal zone, at the University of Otago Portobello Marine Laboratory located on Otago Harbour, Dunedin, New Zealand, for six months. This experiment was replicated to control for seasonality with pigs being placed at the start of the summer (January) and winter season (July) (total *n*=80). Every six weeks, five samples were collected from each zone; the tibia and femur were retrieved and air dried. To ensure consistent and comparable observations, the central bone shaft was chosen as the primary study area because the diaphyseal ends of many bones sustained significant damage. Each sample was scanned three times with an FT-Raman spectrometer, using 1,064nm excitation (1mm spot size, 250mW power). For analysis, particular attention was paid to the five main spectral peaks known to be associated with bone: PO_4^{3-} (960cm^{-1}); CO_3^{2-} (1,070cm^{-1}); CH_2 (1,450cm^{-1}); amide I (1,668cm^{-1}); and amide III (1,246-1,270cm^{-1}).

Using Principal Component Analysis (PCA) and linear discriminant analysis, from the spectral output, quantifiable differences were found between all variables, suggesting that there are both environment and time-specific changes occurring in the surface chemistry of the bone. Environment (inter-tidal and sub-tidal) could be separated with 88%-92% accuracy. In addition, separation by exposure time (6,12,18, and 24 weeks) was possible with 42%-70% accuracy when environment was known. PC loadings showed shifting and broadening of the PO_4^{3-} peak and an increase in CO_3^{2-} substitution into the hydroxyapatite, over time. An increase in CO_3^{2-} substitution creates disorder in the hydroxyapatite. Over time, the amide bands became less defined, indicating the breakdown of collagen. The change in the ratio of organic and mineral components was shown to be a key contributor for differentiating time. The presence of a carotenoid peak (1,525cm^{-1}) in inter-tidal, but not sub-tidal, samples appears to be a significant determinant in separating samples from the two environments and is indicative of algae growth. These results are a major step in better understanding how a marine environment affects juvenile bone and provides important information on the preservational trajectories of juvenile bone in a marine context.

Marine Decomposition, Juvenile Bone, Taphonomy

A130 The Skeletal Histo-Taphonomy of Deep Coastal Marine Submersion and Exposure

Lynne S. Bell, PhD, Simon Fraser University, Dept of Criminology, 8888 University Drive, Burnaby, BC V5A 1S6, CANADA; and Gail S. Anderson, PhD, Simon Fraser University, School of Criminology, 8888 University Drive, Burnaby, BC V5A 1S6, CANADA*

After attending this presentation, attendees will be aware of how pig carcasses, submersed in a cold marine water body, were affected at the microstructural level. Attendees will understand the experimental parameters and be able to recognize the specific "marine change" observed in bony microstructure.

This presentation will impact the forensic science community by explaining the effect of marine exposure to bone and illustrating how to identify associated taphonomic signatures that may be attributed as a result. This adds an important tier of new knowledge to a little-investigated area of forensic anthropology.

The microstructural preservation associated with marine exposure has been the subject of a small number of forensic studies. Work presented here represents the culmination of six deployments in a cold, deep coastal ocean body, the Salish Sea (in southwestern British Columbia and northwestern Washington), one of the world's busiest waterways. Pigs with weights between 16.3kg and 24.5kg, were freshly killed and placed in an experimental rig which deployed two pigs per deployment to the sea floor for an approximate period of six months. Lights and a High-Definition (HD) camera were turned on for a short period of time every 15 minutes per hour for the entire deployment period. Each deployment was at a different locale within the study area at differing times of the year.

Pigs proceeded to skeletonization in all cases, but this reduction did not proceed equally at each site in terms of time. Pigs were reduced within a period of days extending to months. Once recovered, pig bone was stored in seawater and transported within a 12-hour period to the laboratory where it was then washed with cold freshwater to flush out as much salt as possible, and allowed to air dry within the laboratory to stabilize. All pig bone recovered was jet black on recovery and within a 48-hour period would lose this coloration completely, becoming the white color considered normal for bone. The black color would partly be replaced by orange staining to the surface. During this time, bone was macroscopically screened using the ZEISS Stemi microscope. Bone that was untreated prior to freezing was also screened, and in no instance was there any evidence of biofilm. Washed mid-shaft femora and mid-shaft ribs, which had stabilized longer than four months, were prepared for light microscopy and three sequential transverse 80-micron sections were made for each bone using the Leica® microtome SP1600, mounted in **Digital Picture Exchange (**DPX) onto 35mm glass slides, and viewed on a ZEISS Axioscope.A1 using normal and circularly polarized light with a Light-Emitting Diode (LED) light source.

The results indicated that not all deployment environments rendered the marine tunneling observed in other studies; however, this tunneling was observed at different deployment sites and the tunneling dimensions were consistent with those observed by others as a characteristic constrained marine-change. Tunnels consistently had diameters of 5-7 microns and were peripheral to the outer cortex, were never seen on the medullary aspect, nor were they internal to the cortical osteonal systems. The observed orange staining was seen to minimally penetrate bone no deeper than 50-100 microns when present. Often this staining was associated with normal anatomical porosity and also with any postmortem tunneling present. No other microstructural change was observed in the pig bone and the bone had excellent internal preservation.

The results from this study indicate that the observed microstructural change is highly constrained in its morphology and the speed of this change occurred at some point during the six-month submersion period, most likely after soft tissue removal. Other studies have seen the penetrating depth of micro-tunneling extend more than 2mm into bone and dentine but, perhaps due to the short submersion period, such deep tunneling was never observed. Tunneling of other carbonaceous substrates has been speculated to be seasonally cyclical, with endoliths recolonizing as water conditions become favorable. Variation may also relate to rapid sedimentation where skeletal material becomes effectively covered, and this certainly happened with the experimental pigs; however, the change is not necessarily inevitable and further work is necessary to understand why this variation in tunnel depth exists with what is otherwise an important taphonomic indicator of marine submersion and exposure.

Marine, Bone, Taphonomy

A131 Taphonomy of the Perinate Skeleton: Redefining Structural Norms and Building Analytical Models

Christiane Baigent, MSc, Metropolitan State University Dept Sociology/Anthr, PO Box 173362, Campus Box 28, Denver, CO 80217-3362*

After attending this presentation, attendees will better understand the structural and analytical problems associated with preservation and the use of indices for scoring taphonomic change in the perinate skeleton.

This presentation will impact the forensic science community by presenting a modified, high-resolution method for documenting taphonomy in the perinate skeleton, as well as by introducing a novel method for standardizing criteria in the documentation and analysis of taphonomy.

The infant homicide rate in the United States has steadily increased over the past 45 years. The Center for Disease Control (CDC) reported 7.3 infant homicides per 100,000 in 2011 — a 75% increase since 1970.[1] The CDC further reports that the homicide risk is greater in the first year of life than in any other year of childhood before age 18.[1] Because they are easily concealed, difficult to recognize, easily relocated by scavengers, and may be further obscured by taphonomic processes within the post-deposition environment, the preservation of infant remains is often sited as the greatest investigative challenge. Analytical assumptions, such as the presumed "unachievable recovery" of perinate remains has hampered the development of analytical models, critical among which is the documentation of taphonomic change. This study seeks to introduce a modified, zone-based scoring system of indices to document taphonomic change in non-adult bone.

While the methodological frameworks utilized in bioarchaeology and forensic anthropology are often cyclically applicable, current models for analyzing and interpreting taphonomy in non-adult remains are insufficient for forensic investigation. This is largely due to methodological goals; while the bioarchaeologist strives to understand macroscopic socio-biological trends throughout blocks of deep chronological time, the forensic anthropologist is concerned with individualization in the present. Among the most pervading sentiments in the study of non-adults is that their skeletal remains suffer from poor, or a complete lack of, preservation; however, "preservation" is a loaded but often poorly defined term, the use of which may have very different implications in (bio) archaeological and modern context. The distinction between preservation (in the physical structural sense) and transport is critical to the forensic anthropologist who seeks to recover recently deposited remains. Therefore, it is not sufficient for the forensic anthropologist to rely upon simplistic models of preservation, nor should one passively fall back on assumptions associated with loss.

In an effort to quantify taphonomic change, Bello et al. propose the use of three indices to: (1) score the frequency of each bone in a sample (the Bone Representation Index); (2) express the quantity of skeletal material present (i.e., sum of anatomical number of bones) (the Anatomical Preservation Index); and, (3) evaluate the preservation of cortical surfaces as a ratio between sound cortical surfaces and damaged surfaces of each bone (the Qualitative Bone Index).[2,3] A method for increasing the resolution of these indices was developed in an effort to introduce precision and analytical homogeneity to the forensic analysis of the perinate skeleton. This study applied a modified, zone-based scoring system to a sample of 106 skeletons (represented by 371 long bones) from three geologically and temporally distinct archaeological sites within the United Kingdom. Zone scores were applied to two indices (termed the proportional anatomical preservation index and the proportional qualitative bone index). Further, following Waldron's proposal that specific operational definitions be applied to the diagnosis of disease in the skeleton to standardize criteria and facilitate valid comparisons between studies, a model termed "Qualitative Bone Filters" is proposed to serve a similar purpose in the application and documentation of categories of taphonomic change.[4]

The results indicate that "preservation" is highly dependant upon the index applied, and that resolution is effectively increased by the application of zone scores and qualitative filters. Additionally, a general pattern for the progression of taphonomic change commencing at the metaphyses was observed across all three sites. The consistent preservation of the epiphyseal interface was also observed and challenges assumptions surrounding "typical" patterns of degradation. These results indicate that the taphonomy of non-adult remains would be better understood if analytical methods are refined, standardized, and tailored to assess both intrinsic factors, such as the unique physiological and anatomical variables associated with distinct stages of development, and extrinsic factors, such as those inherent to the post-deposition environment.

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Perinate, Taphonomy, Indices

A132 Using Satellite Telemetry to Study Vultures and Other Scavengers in Taphonomic Research

Lauren R. Pharr, PhD, 6634 Central Avenue Pike, Ste 102, Knoxville, TN 37912*

After attending this presentation, attendees will better understand the benefits and limitations of using satellite telemetry and Geographic Information Systems (GIS) in forensic contexts and taphonomic research. Satellite telemetry, or the remote Global Positioning System (GPS) satellite tracking of wildlife, provides location and altitude data on animal movements, such as vulture flight patterns.

This presentation will impact the forensic science community by providing new insight concerning: (1) whether or not vultures can be trained, then tracked as a way to locate human remains; and, (2) the methodological limitations of satellite telemetry when applied to studies involving terrestrial scavengers.

The circling vulture is a universal symbol of death, and these obligate scavengers use olfaction to search for fresh decay. In previous instances where individuals were reported missing, anecdotal accounts have suggested that investigators should search the area where circling vultures were seen; however, the circling vulture may be soaring within a wind vortex, or thermal, rather than about to scavenge a set of decaying remains.

To learn if vulture flight patterns can be used to identify vulture-scavenging locations, six vultures were trapped, GPS tagged, then released from the Texas State Forensic Anthropology Research Facility (FARF) in San Marcos, TX. The hourly locations of the vultures were monitored for six months and resulted in more than 15,000 GPS data points that included information on geographic location (distance), altitude, and flight speed. These variables were analyzed using GIS, allowing for the identification of scavenging behaviors in vultures.

Providing the ability to detect likely vulture scavenging locations from GPS tracking, satellite telemetry provides a wealth of information useful for advancing forensic taphonomy research. Nevertheless, limitations of satellite telemetry should be considered before a tracking study begins. These limitations include costs and logistical difficulties with trapping and tagging animals as seen in the examples below.

In 2014, a Medical Examiner (ME) consulted with this research regarding his plan to trap, train, and then release and track a GPS-equipped vulture to locate human decedents. Because vultures travel long distances, the idea of training a vulture to locate a human body in a fixed, small area is not realistic. For example, turkey vultures released from FARF traveled throughout Texas and into Oklahoma. Moreover, rather than a specific human or animal food source, vultures prefer to locate and scavenge fresh carrion by using their sense of smell. The benefits of the ME's proposal did not outweigh the costs in terms of time or finances.

Forensic anthropologists have suggested that the vulture tracking project be repeated using terrestrial scavengers such as coyotes. Based on experience, this could provide new insight concerning the distance a coyote could potentially travel with a bone from a forensic case; however, detecting the scavenging behavior of a coyote within the GPS data is a concern. For each vulture tracking location, GPS transmitters provided altitude and flight speed. Hourly changes in these two values allowed for the detection of possible scavenging locations; however, a coyote is always close to the ground, which makes it difficult to determine if a coyote is resting or scavenging. Therefore, the information that was most helpful in identifying the patterns in vulture behavior will be absent in terrestrial scavengers. Thus, satellite telemetry will not work as well for land scavengers because of the difficulty in identifying their scavenging behaviors.

Despite the limitations associated with GPS tracking research, with careful planning, a GPS study can provide new insight into scavenging behavior and substantially benefit the forensic community in solving cases for years to come.

This project was funded by the National Science Foundation (NSF), Louisiana State University (LSU) West-Russell Travel Grant, LSU West-Russell Materials Grant, and a Doctoral Dissertation Fellowship provided by the LSU Graduate School. The findings and opinions to be presented are those of the author and not necessarily those of either the NSF or LSU.

The use of migratory birds and other animals for research was conducted with federal, state, and university approval. All vultures were released unharmed and permits are available upon request.

GPS Tracking, Scavenger, Vulture

A133 Postmortem Intervals in Mice Submerged in Aqueous Environments at 20°C

Elizabeth N. Celata, MS, 42 Aldwick Rise, Fairport, NY 14450*

After attending this presentation, attendees will understand the variances in decomposition in aqueous environments such as marine and freshwater. The goal of this presentation is to encourage further academic study and experiments to study the decomposition rates in different water sources and soil samples globally.

This presentation will impact the forensic science community by reiterating and emphasizing the importance of continued research in aqueous environments due to continual changes in various water sources. The data obtained from this study demonstrates the differences in decomposition rate between the River Bourne and the English Channel within Bournemouth, United Kingdom; however, the need for an experiment which spans more than six weeks is noted to explore the possibility of variances increasing to the point of significance after a set point in time.

Aquatic environments offer a unique challenge in determining postmortem intervals. Water sources in the same region — even within a few miles of each other — can differ in salinity and overall mineral composition. Limiting confounding variables in a laboratory environment removes the variance in animal activity as well as temperature and light differences within the same environment due to depth. While aqueous submersion can impede decay in some aspects, submersion can also produce decomposition stages not seen in terrestrial burials. Submerged bodies have a greater likelihood of accelerated skin slippage with extended preservation of fatty tissues. Particular specimens were noted in this study to enter a gelatinous stage as a result of the varying speeds in internal and external decomposition.

To further understand the variances, 54 mice were submerged in marine water, freshwater, and a control environment at 20°C. The two water sources utilized for this study were from the surrounding area around Bournemouth, United Kingdom. The marine water was obtained near the Bournemouth Pier coastline on the English Channel while the freshwater was taken from the River Bourne, which runs into the channel. The 54 mice displayed sequential stages at differing rates over a six-week time period. Regression plots and comparative *T*-tests demonstrated that internal putrefaction rates, weight differences pre- and post-submersion, and abdominal circumference pre- and post-submersion of the aqueous environments differed significantly from the control group. The aqueous subjects did not vary significantly from each other quantitatively; however, the salinity of the marine samples resulted in differences visually which might not have occurred in a deeper container. The postmortem intervals were not consistent regardless of temperature or environment, though a clear variance was noted between the control and the submerged groups.

Aqueous Decomposition, Submerged Specimens, Forensic Science

CRIMINALISTICS

B1 Investigating the Use of MicroRNAs (miRNAs) for the Identification of Forensically Relevant Body Fluids

Kelsie R. Weir, BA, 9124 E Mill Creek Road, Troy, IL 62294; and Claire Glynn, PhD, Henry C. Lee College of Forensic Science, University of New Haven, West Haven, CT 06516*

After attending this presentation, attendees will gain insight into the field of miRNA analysis and its potential application to forensic science, in particular for body fluid identification. This presentation includes extensive research to identify the optimal method of miRNA extraction through to the validation of particular miRNAs previously suggested as markers for particular body fluids.

This presentation will impact the forensic science community by introducing the potential of this novel method for the confirmatory molecular identification of forensically relevant body fluids. This presentation will provide a valuable contribution to the growing field of miRNA analysis for body fluid identification.

MiRNAs are small, non-coding single-stranded RNA molecules, typically 19-25 nucleotides in length. Previously, they were assumed to have no function and were referred to as "junk DNA;" however, they are now known to play crucial roles in many biological processes and have been shown to be highly robust under chemical and physical conditions. Furthermore, through extensive research in the biomedical field, they have also been shown to have high tissue specificity, which infers great advantages to their role in the forensic science field.[1] Due to these qualities, it is proposed that miRNAs could be ideal for the identification of forensically relevant body fluids. The goal of this research was to investigate the ability to extract miRNAs from body fluids using multiple methods, and then to validate miRNAs previously identified to show specificity for particular body fluids using Relative Quantitative Polymerase Chain Reaction (RQ-PCR), which has been shown to be the gold standard for observation of miRNA expression.[2]

Following informed consent, five body fluids were collected from ten volunteers (n=50), including venous blood, menstrual blood, semen, saliva, and vaginal secretions. Each sample was extracted using three different methods including: mirVana™ miRNA isolation kit, miRNeasy® mini kit, and a modified mirVana™ method with Trizol. The manufacturer's instructions of each kit were followed throughout. The quality and quantity of extracted miRNA was determined using an Eon™ spectrophotometer which measures the full spectrum (220nm-750nm) for accurate measurement of concentrations (A260) and protein contamination (ratio A260/280). The concentration of each extracted sample was recorded in ng/µl and the quality assessed by analysis of the 260/280 ratio. RQ-PCR was performed using a 9700 Thermal Cycler and 7900HT Real Time PCR System, targeting six miRNAs of interest, namely miR-451, miR-412, miR-891a, miR-205, and miR-124a, with miR-16 used as the endogenous control. Data analysis was performed using Sequence Detection System (SDS) software and Mintab® 16.0.

The results of this study show that quantifiable miRNA was extracted from each sample; however, remarkable variation was observed in the yields obtained depending on the methods used for each body fluid. Overall, the results suggest the miRNeasy® mini kit provided consistently higher yields throughout all the body fluids, with the exception of saliva, where the modified mirVana™ method proved to be superior. Each miRNA of interest was detectable in the relevant body fluid with significant dysregulation observed across the body fluids for each miRNA.

This study has identified the optimal method for extraction of miRNAs from body fluids and further validates a selection of miRNAs previously suggested as potential biomarkers. This research highlights the overwhelming potential of miRNAs as novel molecular markers for the confirmatory identification of forensically relevant body fluids.

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mircoRNA, Body Fluids, Identification

B2 The Effectiveness of Various Strategies to Improve DNA Analysis of Formaldehyde-Damaged Tissues From Embalmed Cadavers for Human Identification (HID) Purposes

Natalia Czado, MS, 43 Virginia Avenue, Woonsocket, RI 02895; Bobby L. LaRue, Jr., PhD, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107; Amanda Wheeler, BS, 1105 Beasley Hills Lane, Houston, TX 77008; Rachel M. Houston, BS, 3505 Snidow Drive, Plano, TX 75025; Amy E. Sorensen, MSFS, 11 Webb Creek Place, The Woodlands, TX 77382; David A. Gangitano, PhD, Sam Houston State University, 13906 Paradise Valley Drive, Houston, TX 77069; and Sheree R. Hughes-Stamm, PhD, Sam Houston State University, Dept of Forensic Science, Huntsville, TX 77341*

After attending this presentation, attendees will better understand the effectiveness of various strategies to improve DNA typing from severely damaged and degraded Formalin-Fixed (FF) tissue samples for HID purposes.

This presentation will impact the forensic science community by suggesting that, rather than attempting to improve the quantity and quality of severely damaged and degraded DNA template (present in FF samples) prior to Short Tandem Repeat (STR) typing, a more productive approach for HID purposes may be to utilize Insertion/Deletion (INDEL) panels or Single Nucleotide Polymorphism (SNP) markers using Massively Parallel Sequencing (MPS) technologies.

FF tissues for genetic analysis may be obtained from autopsied or archived pathology samples, embalmed cadavers, or repatriated remains. STRs have been the gold standard markers for DNA HID for more than 15 years; however, after treatment with formalin fixatives, many samples are not successfully genotyped using STR analysis. Instead, other methods that pre-amplify the low amount of good quality DNA, repair the damaged DNA template, or use alternate genetic markers to amplify smaller target regions may generate more probative genetic information from these samples.

This study investigated the ability of three different Whole Genome Amplification (WGA) methods (GenomePlex® Complete WGA Kit; Illustra™ Ready-To-Go™ GenomiPhi™ V3 DNA Amplification Kit; and QIAGEN® REPLI-g FFPE Kit) plus one DNA repair treatment to improve downstream STR typing of FF tissues from embalmed cadavers.

In addition, the use of bi-allelic markers, such as INDELs and SNPs, were investigated. These markers, smaller than 200 base pairs (bp) in length, are less susceptible to degradation and therefore may also be more likely to amplify in highly damaged DNA, as in the case of FF tissues. The comparative Random Match Probabilities (RMP) of each sample using STRs, a battery of 39 INDEL markers, and a panel of 124 SNP markers using MPS for HID purposes (HID-Ion AmpliSeq™ Identity Panel) were examined.

This study presents the results of this work, in which none of the three WGA methods or the DNA repair treatment tested in this study consistently yielded more complete STR profiles than the untreated FF samples; however, when the RMPs of each sample obtained using the INDEL and SNP-based MPS panels were compared to those generated from the partial STR profiles obtained from non-treated FF samples, the INDEL and SNP markers generated notably lower RMPs, providing more robust DNA identifications.

Formalin-Fixed, INDELs, Massive Parallel Sequencing

B3 Tertiary Transfer of DNA by Examination Gloves Between Evidentiary Items at Crime Scenes

Marisa Teal Ketchum, BS, University of Indianapolis, 3767 S State Avenue, #210, Indianapolis, IN 46227; Erin L. Vollmer, BA*, University at Indianapolis, 4930 Rue Vallee, Apt 30, Indianapolis, IN 46227; Jenna Carnes, University of Indianapolis, 1400 E Hanna Avenue, Indianapolis, IN 46227; Krista E. Latham, PhD, University of Indianapolis, Biology Dept, 1400 E Hanna Avenue, Indianapolis, IN 46227; Cynthia Cale, BS, Strand Diagnostics, 5770 Decatur Boulevard, Ste A, Indianapolis, IN 46241; and Gay L. Bush, PhD, Strand Diagnostics, 5770 Decatur Boulevard, Ste A, Indianapolis, IN 46241*

After attending this presentation, attendees will better understand the potential for tertiary transfer of DNA among evidentiary items at a crime scene via examination gloves.

This presentation will impact the forensic science community by demonstrating potential routes for the cross-contamination of items collected during the course of processing a crime scene.

The increasingly high value placed upon DNA evidence has catalyzed the optimization of more sensitive equipment and chemistries utilized in the detection and analysis of DNA. While these advancements allow smaller quantities of DNA obtained from evidentiary items to be analyzed, they also increase the risk of detecting extraneous DNA that may not be associated with the criminal act. The process of collecting evidentiary items at the crime scene is one potential route of contamination. Protocols for evidence collection in the field, including how frequently gloves should be changed while processing a crime scene, vary among crime laboratories and personnel. The potential exists for the technician to transfer DNA among the items being collected at the crime scene via their examination gloves if not frequently changed.

The main learning objective of this pilot study is to investigate the possibility of transferring DNA among evidentiary items and to evaluate whether or not the amount of transferred DNA that is detected could complicate subsequent genetic analyses and interpretations. It is hypothesized that: (1) examination gloves can act as a vector for the transfer of DNA from one item to another; (2) the quantity of DNA transferred among items will decrease with each subsequent handling; and, (3) the quantity of DNA detected on each item will be at a level that is great enough to compromise the interpretation of DNA typing results.

A sterile plastic cup was handled by ungloved hands. A gloved laboratory technician handled this item (Tier 1) to swab for subsequent DNA analysis. The laboratory technician then handled a second sterile and untouched cup (Tier 2) without changing gloves, followed by a third cup (Tier 3). These items were also swabbed to detect any DNA that might have been transferred to the various cups during the handling process. This procedure was repeated a total of seven times with the laboratory technician changing gloves in between each trial. DNA extraction was conducted using the QIAGEN® QIAamp® DNA Mini Kit. The Quantifiler® Human DNA Quantification Kit in conjunction with an Applied Biosystems® 7500 Real-Time Polymerase Chain Reaction (PCR) instrument was used to estimate the quantity of human DNA present in each sample. Amplification was performed by PCR on an Applied Biosystems® 9700 thermal cycler. Amplified product was analyzed using capillary electrophoresis on an Applied Biosystems® 3130xl (16 capillary) instrument in conjunction with GeneMapper® ID (version 3.2.1).

Primary transfer DNA was detected on all Tier 1 cups, demonstrating the ease of transfer of DNA from an individual to an object. DNA was transferred to the technician's examination gloves and to the Tier 2 cups in 67% of the trials. A partial profile was obtained from one Tier 3 cup, which had the potential to interfere with interpretation. These results demonstrate the vulnerability of evidentiary items to cross-contaminate if examination gloves are not frequently changed at the crime scene. Therefore, the first hypothesis cannot be rejected. The second hypothesis cannot be rejected because the quantity of DNA detected on the items decreased as the laboratory technician handled the Tier 2 and Tier 3 items. Finally, the third hypothesis cannot be rejected since the alleles detected on the Tier 2 and Tier 3 items would interfere with the interpretation of the DNA profile results.

The results of this experiment demonstrate that although tertiary transfer did not occur in every trial, there is the potential for the cross-contamination of evidentiary items via examination gloves. The potential for tertiary transfer in the field should warrant caution during the handling of evidence due to the possibility of transferring an individual's DNA to an item not actually touched by that person. Crime laboratories and personnel should implement procedures to negate this possible route of contamination.

DNA, Tertiary Transfer, Examination Gloves

B4 Optimization and Validation of the ForensicGEM™ Rapid Extraction Method for High-Throughput Processing of Cotton Buccal Swabs

Kyleen Elizabeth Elwick, BS, 1079 Ridgecrest Drive, Goodlettsville, TN 37072; Sheree R. Hughes-Stamm, PhD, Sam Houston State University, Dept of Forensic Science, Huntsville, TX 77341; Kimberly S. Andreaggi, MFS, ARP/AFDIL, 115 Purple Heart Drive, Dover AFB, DE 19902; and Michelle A. Peck, MFS, Armed Forces DNA Identification Laboratory, 115 Purple Heart Drive, Dover AFB, DE 19902*

After attending this presentation, attendees will understand the benefits of using forensicGEM™ as an optimal extraction method for the High-Throughput (HTP) processing of reference samples.

This presentation will impact the forensic science community by demonstrating an HTP, rapid extraction method that decreases the time and cost of extraction. By removing the need for lengthy extraction protocols, this method will assist in the elimination of reference backlogs in forensic laboratories.

Most extraction methods are time consuming and have a high risk for contamination due to numerous steps involved in the purification of the DNA. The Armed Forces DNA Identification Laboratory (AFDIL) currently uses DNA IQ™ for HTP processing of cotton buccal swabs, which is expensive and time consuming. The forensicGEM™ method uses a proteinase from the thermophilic *Bacillus* sp. EA1 and robust buffers to perform a single-step extraction in approximately ten minutes without the need for purification. ForensicGEM™ chemistry is rapid, compatible with downstream processing methods, adaptable to tubes or plates, amenable to automation, and inexpensive. The ability to do a single-step extraction without purification will facilitate the rapid low-cost HTP processing of cotton buccal swabs.

For use at the AFDIL, the forensicGEM™ Storage Card (Saliva) kit was optimized and validated for HTP processing of cotton buccal swabs. The optimization experiments examined three steps in the procedure: (1) cell elution; (2) eluate input volume; and, (3) extraction buffer volume. First, the five-minute elution step, which is performed in a ThermoMixer® C to dislodge the buccal cells from the swabs, was evaluated at two different speeds: 900rpm and 2,000rpm. These two conditions were each tested on 90 buccal samples to assess DNA yield and the impact of the swab position on the 96-well plate. Agitating samples at 2,000rpm corresponded to a 79% success rate (full profiles) when typed with AmpFℓSTR Yfiler®, whereas agitating swabs at 900rpm demonstrated only a 46% success rate. Plate location of the buccal swab appeared to make no difference in the success of profiles. Based on these results, 2,000rpm was determined to be the optimal elution condition.

Next, the volumes of the eluate input and extraction buffer were also investigated to further improve DNA yield. Cotton buccal swabs were collected from a total of 15 sources (in duplicate) and were extracted using five extraction conditions, testing a range of 40µL-100µL of eluate with a range of 10µL-25µL of buffer. The extracts were evaluated with several commercially available Short Tandem Repeat (STR) kits as well as the Control Region (CR) of the mitochondrial DNA (mtDNA) genome. The extraction condition that used 70µL of eluate input with 10µL of forensicGEM™ buffer outperformed the other conditions, generating at least a 90% first pass success rate with CR amplification and all STR kits. The STR profiles were of high quality (e.g., optimal peak heights, low intracolor imbalance) and required minimal reprocessing. This performance mimicked current processing methods and was therefore selected as the optimized protocol. Further testing is currently being performed to validate the optimized forensicGEM™ method for sensitivity, repeatability, and reproducibility according to the Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines using quantitative Polymerase Chain Reaction (PCR), STR typing, and mtDNA analysis. Implementation of this optimized HTP extraction will allow for increased throughput capabilities by reducing both costs and processing time by approximately 65%.

The opinions or assertions presented herein are the private views of the authors and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the United States Army Medical Research and Materiel Command, or the Armed Forces Medical Examiner System.

Rapid Extraction, DNA Databasing, ForensicGEM™

B5 Investigating Simultaneous Extraction of RNA and DNA From Forensically Relevant Body Fluids

Sarah L. Markland, 300 Boston Post Road, West Haven, CT 06516; Kelsie R. Weir, BA, 9124 E Mill Creek Road, Troy, IL 62294; and Claire Glynn, PhD, Henry C. Lee College of Forensic Science, University of New Haven, West Haven, CT 06516*

After attending this presentation, attendees will be aware of the potential of the simultaneous extraction of microRNA (miRNA) and DNA from within a single sample for forensically relevant body fluids. This includes the investigation and comparison of two commercially available co-extraction kits for the yields obtained from each kit, the sensitivity of each kit, and the ability to obtain both miRNA and DNA profiles.

This presentation will impact the forensic science community by highlighting the usefulness of co-extracting RNA and DNA from a single sample. This method could lead to both confirmatory body fluid identification and human DNA profiling.

MiRNAs are a class of short non-coding RNA molecules which are approximately 22 nucleotides in length and regulate the post-transcriptional gene expression in many eukaryotes. It has recently been suggested that miRNAs could serve as potential biomarkers for body fluid identification in forensic investigations. Previously, messengerRNA (mRNA) was proposed as a useful tool for body fluid identification; however, due to their instability and susceptibility to degradation, they have not shown great promise in the forensic field. In contrast, miRNAs are remarkably robust and infer great stability due to their small size; however, depending on the volume of sample available, the miRNA analysis could consume the sample and potentially eliminate the ability to obtain a DNA profile. Therefore, there is a great need for a method which performs both RNA and DNA extraction simultaneously from one sample. There have been limited advancements in this area with only a few commercial methods currently offered for this purpose. Little research has been performed on this topic in the forensic setting. The goal of this study was to investigate two commercially available co-extraction RNA/DNA kits by examining the quality and quantity of RNA/DNA extracted, followed by a series of dilutions to test sensitivity, and finally by the ability to obtain a miRNA signal and DNA profile from a range of forensically relevant body fluids.

Following ethical approval from the Institutional Review Board and informed volunteer consent, venous blood, semen, saliva, and urine were collected from five volunteers (n=20). The two commercially available kits that were investigated were the Zymo Research ZR-Duet™ DNA/RNA MiniPrep kit and the QIAGEN® AllPrep™ DNA/RNA Mini Kit. The manufacturer guidelines were followed for each kit. First, neat samples were extracted from 200µl of each body fluid using each kit. Following this, a series of dilutions of each body fluid were created in 1:2, 1:10, 1:25, and 1:50 ratios and then the co-extraction of miRNA and DNA were performed using the same kits. Following RNA/DNA extraction, all eluates were analyzed to determine the RNA/DNA concentration in each sample. This was achieved using Biotek Eon™ spectrophotometer which measures the full spectrum (220nm-750nm) for accurate measurement of concentrations (A260), protein contamination (ratio A260/280), and contamination with buffer components or organic compounds (ratio A260/A230). The concentration of each extracted sample was recorded in ng/µl and quality assessed by analysis of the 260/280 ratio. In the final step, Relative Quantitative Polymerase Chain Reaction (RQ-PCR) was performed on a selection of the RNA samples targeting miR-16 to determine if a miRNA signal was present within the extract. In parallel, STR analysis was performed on a selection of the DNA samples to obtain full human DNA profiles.

The results showed that quantifiable amounts of both DNA and RNA were obtained in the neat samples of all the body fluids using both kits; however, the concentrations obtained were highly variable depending on the particular body fluid and the particular kit used. The results obtained from the diluted samples produced varying concentrations at much lower levels, as expected. Overall, the Zymo Research kit proved to obtain higher concentrations of both DNA and RNA when compared to the QIAGEN® kit. Finally, miRNA signals and full DNA profiles were obtained from all samples selected for miRNA/DNA profiling.

In conclusion, this study reveals the ability to successfully co-extract both RNA and DNA from forensically relevant body fluids, suggesting the Zymo Research kit as a superior method for this purpose. This research highlights the potential of miRNAs for the identification of forensically relevant body fluids as it has shown to be possible the ability to extract both miRNA profiles and DNA profiles from a single sample, which could prove crucial to a forensic investigation.

MicroRNA, DNA, Co-Extraction

B6 Investigating the Use of Raman Spectroscopy for the Differentiation of Mixed Body Fluid Samples

Tyler J. Schlagetter, 441 Lunar Street, Sidney, OH 45365; Brooke W. Kammrath, PhD, University of New Haven, Forensic Science Dept, 300 Boston Post Road, West Haven, CT 06516; and Claire Glynn, PhD, Henry C. Lee College of Forensic Science, University of New Haven, West Haven, CT 06516*

After attending this presentation, attendees will be aware of the potential of Raman spectroscopy to be integrated into forensic body fluid analysis. This includes its use in both identifying individual fluids, differentiating between fluids in mixed samples, and analyzing samples found on different substrates.

This presentation will impact the forensic science community by discussing the advantages and limitations of Raman spectroscopy with regard to its use in the identification and analysis of body fluids and their mixtures.

In forensic investigations, stains recovered from crime scenes can often be a combination of different body fluids (e.g., semen and saliva, or blood and saliva). With the success of forensic DNA typing over the past three decades, there are several strategies for the successful resolution of DNA mixtures; however, there has been little research into effective analytical methods for the resolution of mixtures of body fluids. While there are certain methods currently employed for the confirmatory identification of body fluids, many of these are destructive (in terms of consuming the sample), some have variable results and are labor intensive, while none have the capability of separating two body fluids in one sample. The non-destructive capabilities of Raman spectroscopy have allowed it to become a growing source of interest in the forensic science profession. Research has shown that Raman spectroscopy produces spectra which can be used to identify blood, semen, saliva, vaginal secretions, and sweat without consuming the sample.[1] The goal of this study was to investigate the use of Raman spectroscopy to identify the individual body fluids comprising a mixed body fluid sample.

Following informed consent, venous blood, semen, saliva, and urine were collected from five volunteers (n=20). Raman spectroscopy with a 785nm excitation wavelength under controlled laboratory conditions was performed on the individual body fluids, followed by body fluid mixtures of varying ratios. The body fluids were also tested on a variety of substrates (aluminum slide, black cotton, and white cotton). Last, DNA profiling was performed on a selection of the scanned samples to investigate the ability to obtain a DNA profile post-Raman analysis.

The results showed that each of the body fluids produced their own individual spectra. Mixture testing revealed that detection of both body fluids was possible with blood and semen, saliva and semen, saliva and urine, and semen and urine mixtures; however, the results indicated that the blood gave too strong a Raman signal to allow for saliva or urine to be fully detected. The substrate analysis revealed that blood was the only body fluid partially detected and was only detected on the white cotton. All mixtures tested on the substrates gave indefinite results due to interference from the substrates. Full DNA profiles were obtained from all samples tested.

This study reveals the successful use of Raman spectroscopy for the resolution of a number of mixed body fluid samples and highlights the potential of the technique to be introduced as a novel, non-destructive method for the identification of forensically relevant body fluids.

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Raman, Mixtures, Body Fluids

B7 The Identification of Biological Fluids Based on DNA Methylation Differences Using High Resolution Melt Curve Analysis

Susan Cheng, BS, 100 College Drive, Allentown, PA 18104; and K. Joy Karnas, PhD, Cedar Crest College, 100 College Drive, Allentown, PA 18104*

After attending this presentation, attendees will understand how high resolution melt curve analysis may be used to distinguish between biological fluids using DNA methylation patterns that differ between cell types.

This presentation will impact the forensic science community by providing a novel method of potentially distinguishing menstrual blood and peripheral blood, as well as differentiating deposited vaginal fluid, saliva, and semen stains. In addition, methylation analysis uses the same DNA extraction protocol as Short Tandem Repeat (STR) typing, making these two methods compatible for side-by-side analysis.

Identifying the origin of the biological fluid is important in crime scene reconstruction and provides information to link evidence to the crime. Traditional methods of identifying body fluids rely on proteins for identification and are initially presumptive in nature. Presumptive tests may not be reliable due in part to cross reactivity with other biological fluids, resulting in false positives. In addition, false negatives may result due to degradation of protein antigens by heat and humidity. Once a stain presumptively tests positive for a biological fluid, an additional confirmatory test is necessary in order for the evidence to have legal and scientific standing in a court of law. Due to time and budgetary constraints as well as insufficient amounts of sample, confirmatory assays are not always able to be performed by forensic laboratories.[1] DNA methylation markers and pyrosequencing have recently been evaluated as an alternate method for body fluid identification. While these studies were able to discriminate the specific body fluid, pyrosequencing is expensive and uses equipment that is not available in most forensic laboratories.[2]

This study is a continuation of previous research that differentiated semen from other body fluids using DACT1 primers.[3] In this study, menstrual blood, blood, vaginal fluid, saliva, urine, sweat, and semen samples were obtained from volunteers by self-collection. An organic extraction was used to isolate DNA from each swab prior to subjecting the DNA to bisulfite conversion, which converts unmethylated cytosines to uracil, using a Zymo Research EZ DNA-Methylation-Lightning™ kit. High resolution melt curve analysis was carried out to analyze the melting temperature of the amplicons containing known sites of differential DNA methylation patterns using the QIAGEN® EpiTect™ High Resolution Melt Polymerase Chain Reaction (PCR) Kit. Primers used in this study are either proprietary or previously published.

Results from this study indicate that the HOX-B6 primer distinguished four out of ten menstrual blood samples from circulating blood samples. The primer also distinguished five out of ten vaginal fluid samples from other biological fluids (including blood, saliva, urine, and sweat). Using the SOX2OT primer, seven out of ten saliva samples were differentiated from other biological fluids (including menstrual blood, blood, vaginal fluid, urine, and sweat). This indicates that when samples are analyzed in tandem using the HOX-B6, SOX2OT, and DACT1 primers, menstrual blood, vaginal fluid, saliva, and semen may be differentiated from other body fluids.

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2. Park J.L., Kwon O.H., Kim J.H., Yoo H.S., Lee H.C., Woo K.M., Kim S.Y., Lee S.H., Kim Y.S. Identification of body fluid-specific DNA methylation markers for use in forensic science. *Forensic Sci Int Genet* 2014;13:147-153.
3. Deppen C. High-resolution melt curve analysis of DNA methylation status as a novel method for human semen identification. *Proceedings of the American Academy of Forensic Sciences*, 67th Annual Scientific Meeting; Orlando, FL. 2014.

Forensic Science, DNA Methylation, Biological Fluids

B8 Analysis of Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR) Spectra to Differentiate Menstrual and Venous Blood on Various Substrates

Alicia Quinn, BS, 120 Stoney Meadow Lane, Madison Township, PA 18444; and Kelly M. Elkins, PhD*, Towson University, Chem Dept & Forensic Science Program, 8000 York Road, Towson, MD 21252*

After attending this presentation, attendees will better understand ATR/FTIR spectroscopy and how this method can be used to analyze spectra for body fluid samples on various substrates and differentiate venous and menstrual blood following statistical analysis.

This presentation will impact the forensic science community by suggesting a rapid, non-destructive method for differentiating body fluid samples, including venous and menstrual blood, on various substrates that can provide significant discriminatory information for forensic comparisons.

Body fluids are often encountered at crime scenes, especially those in which sexual and violent crimes have been committed. An alternate light source is often used to locate the presence of body fluids but is not used to identify them. Chemical methods are typically employed to tentatively identify the presence of body fluids including blood, semen, saliva, urine, and fecal matter. A drawback of using chemical methods is that they may dilute the sample, cause degradation, or interfere with DNA typing. ATR/FTIR spectroscopy has been suggested as a method that can be used to identify and differentiate body fluids in simulated forensic samples; however, only neat samples and samples on white paper and white cotton substrates were tested.

This study evaluated the entire ATR/FTIR spectrum (including the amide banding and fingerprint regions) for the body fluid samples on several substrates including colored cotton, polyester, nylon, wood, and glass. Blood, semen, and breast milk standard samples were purchased from Lee™ Biosolutions. Saliva and menstrual blood samples were obtained from healthy donors in accordance with Towson University Institutional Review Board (TU IRB) approval. A Thermo Fisher Scientific Nicolet™ iS™10 spectrometer with a Smart iTR™ ATR attachment and equipped with the Omnic™ software version 32 was used to collect all spectra. ATR/FTIR absorbance spectra (128 scans, 4,000 to 400cm-1 spectral range, 1.929cm-1 spectral resolution) were recorded using the ATR diamond crystal in ambient temperature with air as a background. A standardized volume of 20µL of each type of body fluid was deposited onto parafilm and dried at room temperature. The residue layer was applied directly to the cleaned diamond crystal surface. Separately, the neat body fluid samples were pipetted (20µL each) onto five different substrates. Reference spectra were recorded of each substrate and subtracted to identify contribution from the body fluid. Three replicates of each fluid were performed. The average signal was averaged over all scans and the data was saved as a .csv text file for import into Microsoft® Excel® for spectral analysis. Statistics were used in order to differentiate the body fluids from one another by looking at the presence or absence of a particular wavenumber (cm-1) as neat samples and on the surfaces. The statistical technique emphasizes the variations and draws out strong patterns in the dataset. Variations of the body fluid spectra provide significant discriminatory power and provide support for ATR/FTIR to be investigated further as a method that could be employed at the crime scene.

ATR/FTIR, Venous Blood, Menstrual Blood

B9 Touch DNA Recovered From Fired and Unfired Shotgun Shells

Anthony J. Saitta, 57 Guernsey Lane, New Milford, CT 06776; and Peter R. Valentin, MSFS, University of New Haven, Forensic Science Dept, 300 Boston Post Road, West Haven, CT 06516*

After attending this presentation, attendees will better understand the potential implications of touch DNA as it relates to cartridge casings and the impact of the firing process on the ability to recover DNA.

This presentation will impact the forensic science community by providing statistical data from a well-controlled experiment, thus increasing the information gathered on the applications of touch DNA. With more sensitive DNA testing, this information will help redefine the areas and objects that can be tested for DNA.

Over the last decade, major advancements have been made in the field of touch DNA recovery; however, some challenges still exist. In particular, the recovery of touch DNA from firearm cartridge casings has long been regarded as a difficult task with minimal yields expected. This project was designed and conducted to generate valid statistical data on the recovery of touch DNA from both fired and unfired shotgun shells. Modeled after similar experiments, the goal of this study was to expand the data gathered on touch DNA and its applications. This study hypothesized that there would be a higher yield of DNA recovery from plastic shotgun shells than from metallic pistol or rifle cartridge casings. Based on the principles of organic compound interactions, fingerprint oil is expected to adhere better to the low-density polyethylene of the shotgun shell compared to metals such as brass or copper, thus leaving behind more skin cells to be collected and DNA to be extracted. Additionally, it was hypothesized that the unfired shells would yield a higher recovery of DNA, as they were not exposed to the extreme heat generated during the firing process.

In this experiment, 90 12-gauge shotgun shells were collected and examined for the presence of touch DNA. Three participants each loaded and fired 15 rounds, then loaded and ejected 15 rounds without firing. The shells were loaded each time in groups of five, carefully ejected directly into pre-labeled evidence bags, and immediately sealed. Under sterile laboratory conditions, all samples were swabbed using the double-swabbing method: one sterile cotton swab wet with sterile water, followed by one dry sterile cotton swab. These swabs were then extracted using a QIAGEN® Investigator® extraction kit, followed by 40 cycles of Polymerase Chain Reaction (PCR) amplification. After quantification, approximately 17.78% of the unfired samples and 18.60% of the fired samples extracted and quantified yielded sufficient DNA for a partial or full profile. Results of a previous project stated the recovery rate of DNA from brass casings, both fired and unfired, was approximately 36%, while nickel-plated pistol cases only yielded 12%, fired and unfired. Therefore, the results from this study, revealing approximately 18.2% recovery from plastic shotgun cartridges, provides a valuable contribution to the investigation of touch DNA recovery from firearm cartridge casings.

This study gathered controlled statistical data on the recovery rate of touch DNA from fired and unfired shotgun shells, increasing the knowledge of touch DNA and its new potential areas for testing.

Touch DNA, Shotgun, DNA

B10 Use of Massively Parallel Sequencing to Assist With Deconvolution of Short Tandem Repeat (STR) Mixture Profiles

Kelly Grisedale, PhD, Western Carolina University, 111 Memorial Drive, NSB 231, Cullowhee, NC 28723; Jessica Bradley, BS, Western Carolina University, 111 Memorial Drive, NSB 231, Cullowhee, NC 28723; Brittania J. Bintz, MSc, 111 Memorial Drive, NSB 231, Cullowhee, NC 28723; and Mark R. Wilson, PhD, Western Carolina University, Dept of Chemistry/Physics, Forensic Science, Cullowhee, NC 28723*

After attending this presentation, attendees will better understand how DNA samples amplified with traditional fluorescence-based STR kits can be further interrogated using Massively Parallel Sequencing (MPS) to assist with interpretation of low template or mixed DNA profiles.

This presentation will impact the forensic science community by providing an avenue to gain maximum information from mixture samples that are traditionally difficult to interpret with standard DNA profiling techniques.

Forensic DNA profiling by Polymerase Chain Reaction (PCR) of STR is considered a robust and reliable method of human identification. STRs are highly discriminating, and the exponential amplification of the PCR allows for complete profiles to be obtained from as little as 200pg of DNA; however, difficulties can arise in profile interpretation when the starting DNA template is extremely low or if the profile contains DNA from more than one contributor.

Mixture DNA profiles can be especially difficult to interpret when the contributors share alleles. In cases where one individual in the mixture contributes considerably more genetic material than the other individual, the major contributor can mask the profile from the minor contributor, making it nearly impossible to deconvolute shared alleles.

Current methods of STR analysis only provide information regarding the STR fragment length; however, sequencing of STRs could provide further information to assist with deconvolution of mixture profiles. Initially, reads attributed to each STR locus are sorted based on flanking sequences adjacent to the targeted repeat. Repeats contained within each read are counted by the software, and resulting counts are used to calculate an approximate mixture ratio, enabling parsing of alleles to each contributor. Shared alleles can also be deconvoluted. STR fragments may contain Single Nucleotide Polymorphisms (SNPs) that would not be detected with traditional Capillary Electrophoresis (CE) genotyping analysis. Furthermore, many STRs have been shown to have different configurations of repeated units for the same allele.

This presentation proposes a method to use MPS methods to sequence PCR product generated using a commercial STR kit designed for traditional CE analysis. While STR kits designed specifically for MPS are in development, the ability to sequence previously amplified samples could provide further insight into profiles that were difficult to interpret with STR length information alone.

Single-source and two-person mixture samples of various mixture ratios were amplified with the GlobalFiler™ PCR amplification kit. All samples were run on the 3500xL Genetic Analyzer to obtain reference electropherograms. The same PCR product was then prepared for MPS using enzymatic library preparation via tagmentation by Nextera® XT followed by deep sequencing on the Illumina® MiSeq®. Data were then analyzed using STRait Razor software.

Preliminary results indicate that the Nextera® tagmentation step successfully removes the fluorescent tags incorporated into the PCR products during amplification with the GlobalFiler™ kit. This is essential for successful sequencing on the MiSeq®, since this sequencing-by-synthesis method involves fluorescence detection. Furthermore, analysis of the STR sequences in mixed DNA profiles has revealed some different STR sequence motifs for shared alleles that are kept in relatively similar proportions as would be expected from the ratio of input DNA from the two contributors. This has allowed some shared alleles that were initially masked in the reference electropherogram to be assigned to the major or minor contributor.

Massively Parallel Sequencing, Short Tandem Repeats, Mixtures

B11 Absolute Quantitation of Semen-Specific Biomarkers From Post-Coital Samples

Catherine O. Brown, BA, Arcadia University, 450 S Easton Road, Glenside, PA 19038; Masha Signaevsky, BS, Arcadia University, 423 Roslyn Avenue, 2nd Fl, Glenside, PA 19038; Heather E. McKiernan, MSFS, Center for Forensic Science Research & Education, 2300 Stratford Avenue, Willow Grove, PA 19090; Kevin M. Legg, 2300 Stratford Avenue, Willow Grove, PA 19090; and Phillip Danielson, PhD, Department of Biology, 2101 E Wesley Avenue, Lab 223, Denver, CO 80210*

After attending this presentation, attendees will better understand the use of a multiplex tandem mass spectrometry assay for the detection of seminal fluid protein biomarkers that will be of benefit in the determination of the post-coital interval for semen detection.

This presentation will impact the forensic science community by providing results from a quantitative study in an area of forensic science that is under-researched. The results of this study have the potential to aid in determining the post-coital interval, which can further assist sexual assault forensic examiners in the timely collection and processing of sexual assault evidence.

Sexual assault is a prevalent crime in today's society; however, the forensic testing utilized for sexual assault evidence is costly and time consuming, resulting in a backlog of untested evidence. The current enzyme-activated and antibody immunochromatographic tests used by forensic examiners for the identification of seminal fluid only provide presumptive results. This is due to false positives with non-biological material, cross-reactivity, and positive results with non-target fluids. Immunochromatographic tests rely on the tertiary structure of proteins in order to produce positive results; however, proteins may be subjected to undesirable conditions resulting in the unfolding of the tertiary structure, preventing enzyme or antibody interaction. A negative serological test may prevent the sample from receiving additional DNA testing, regardless of whether the protein was present in a degraded form. The presumptive nature of these tests has deterred scientists from reliably determining a post-coital interval. The development of a confirmatory technique for the identification of seminal fluid would allow for the post-coital detection of semen to be determined and would assist in the analysis of sexual assault evidence.

Prior experiments have identified, developed, and validated a qualitative, mass spectrometry-based assay for the confirmatory identification for human seminal fluid. A previously performed sensitivity study has shown that seminal fluid biomarkers (prostatic acid phosphatase, prostate specific antigen, and semenogelin) are readily detectable by mass spectrometry from a little as 1nL of semen, levels at which the currently employed presumptive tests begin to fail.

The goal of the current research was to validate a quantitative variation of the previously developed method in order to quantitate these semen-specific proteins in post-coital samples. The current method employs scheduled multiple reaction monitoring on an Agilent® 6430 triple quadrupole mass spectrometer coupled with a nanoflow chip cube High-Performance Liquid Chromatography (HPLC). Using synthetic peptide standards, a linear calibration model was developed and mock casework samples were used to analyze the multiplex assay's sensitivity and limits of detection in addition to a comparison of the specificity and selectivity of this approach to immunochromatographic assays.

In conclusion, this study provides evidence that mass spectrometry produces more sensitive results for the detection of seminal fluid, providing a more reliable method for the detection of semen in sexual assault samples. Furthermore, the use of mass spectrometry has the potential to enhance the serological screening process and aid in the determination of the post-coital interval.

Forensic Science, Proteomics, Seminal Fluid

B12 Rapid Direct Polymerase Chain Reaction (PCR) of a Y-Chromosomal Short Tandem Repeat (Y-STR) Multiplex as a Screening Tool for the Presence of Male DNA

Bruce R. McCord, PhD, Florida International University, Dept of Chemistry, University Park, Miami, FL 33199; and Georgiana C. Gibson-Daw, MS, 8920 NW 8th Street, Apt 518, Miami, FL 33172*

After attending this presentation, attendees will better understand how the use of rapid direct PCR along with microfluidic separation and specially designed Y-STR multiplexes can screen crime scene samples for the presence of male DNA and thus aid in the analysis of time-sensitive cases.

This presentation will impact the forensic science community by providing results that show how the amount of time and reagents used can be vastly decreased with a combined rapid and direct PCR screening method on which little research has been performed. This presentation will add to the body of research being carried out in forensic DNA analysis that focuses on reducing the amount of time and steps involved in sample analysis. This would enable more samples to be run in a determined amount of time, thus increasing throughput. In addition, the removal of the extraction step would decrease the potential for sample loss and in-laboratory contamination, both of which are important problems when dealing with samples from a crime scene.

It is often extremely important to rapidly screen crime scene samples and unknown individuals who may have been involved in a crime, namely in situations where many samples may need to be run for sorting through excessive amounts of evidence or before detention of a suspect is possible. Examples include seized evidence potentially linked to a suspect or the determination of which blood stains present at a crime scene may be probative.

Mass disasters in particular create a need for rapid, inexpensive screening of DNA samples with a minimum of sample pretreatment. Recently developed DNA typing methods provide the best biometric information for identity, kinship, and geographical origin, but they are not sufficiently fast to permit the detection of a suspect's DNA in real time. Rapid direct PCR procedures can greatly accelerate the processing time because no extraction is necessary.[1-6] This decrease in processing time and reagent volumes leads to a quick turnaround and inexpensive processing of larger numbers of samples. Such procedures have previously been designed for STRs.[7, 2-3] The goal of this project is to develop a rapid and direct method for profiling Y-STRs as a fast and effective screening tool to determine the presence of male DNA in collected samples.

To do this, specially engineered enzymes, high speed thermal cyclers (capable of running 28 cycles in less than 14 minutes), and microfluidic chip-based electrophoresis will be implemented to process a specifically designed Y-STR multiplex.[1, 8-14] The goal is to reduce the analysis time to less than 25 minutes.[7] The designed multiplex includes four Rapidly Mutating (RM) Y-STRs (DYS526a/b, DYS576, DYS626, and DYS570) between 137bp and 402bp in size, with mutation rates of 10^{-2} per meiosis or greater. By using off-the-shelf instruments and commercially available enzymes, it is possible to create a procedure that acts as a quick, highly informative sample-screening process that also retains sufficient DNA for later manual processing using standard STR or Y-STR kits.

In the first phase of this study, a 4-locus Y-STR multiplex was designed and utilized on a conventional 310 Capillary Electrophoresis (CE) and a beta version of a denaturing Microfluidic Electrophoresis (ME) system. This was tested on control DNA standards 2800M Control DNA and HY DNA as well as with donated saliva samples from five adult males. The multiplex was then analyzed using a rapid PCR protocol, using a variety of rapid polymerases in an effort to optimize the speed and balance of the amplification. This procedure was further optimized with the use of Z-Taq and a direct PCR buffer (Any Direct F buffer) to obtain a rapid direct PCR method, which when coupled with microfluidic separation cuts down sample analysis time to less than 40 minutes, with the possibility of decreasing this further.

The results of this study demonstrate the application of rapid direct PCR for the analysis of Y-STRs for evidence screening. Because the process utilizes a small set of rapidly mutating Y-STR loci, it can also provide useful preliminary data on the presence of male DNA for use in suspect identification.

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Rapid PCR, Direct PCR, Microfluidic Y-STR Analysis

B13 Determining the Most Efficient Location for Collecting DNA Samples From Hand Guns

Kaitlyn M. Redman, BS, 190 Robin Ridge Road, Feeding Hills, MA 01030; Kathryn E. Hoodenpyle, MS, 425 E Phelps Street, Springfield, MO 65806; Jill Therriault, BS, Connecticut Department of Emergency Services, Division of Scientific Services, 278 Colony Street, Meriden, CT 06451; Arielle Van Deusen, BS, CT Department of Emergency Services, Division of Scientific Services, 278 Colony Street, Meriden, CT 06451; Jessica Best, MSFS, Connecticut Forensic Lab, 278 Colony Street, Meriden, CT 06451; and Michael S. Adamowicz, PhD, University of New Haven, Dept of Forensic Science, 300 Boston Post Road, West Haven, CT 06516*

After attending this presentation, attendees will better understand the most effective locations to collect samples from hand guns for forensic DNA testing. This study presents data regarding the quantity and quality of DNA recovered from discrete locations on semi-automatic and revolver-style hand guns. It also presents data contrasting the collection of DNA from "overall" swabbing of a firearm versus targeted area collections.

This presentation will impact the forensic science community by providing further experimental data regarding the optimal strategy to use when collecting samples from hand guns for DNA analysis. This data will assist analysts with targeting the areas on hand guns that are most likely to yield the highest quantities of DNA for Short Tandem Repeat (STR) analysis while avoiding collecting wasteful extra samples that show poor results.

DNA recovery from firearms was evaluated in order to determine the most efficient location(s) for the collection of DNA evidence. Previously cleaned firearms (Glock® 19, Beretta® 92, Smith & Wesson® 10-5, and Taurus® Ultra-Lite) were handled for approximately one hour, including having the actions repeatedly worked and then discharged prior to sample collection using a double-swab method (moistened and dry). Results from the individual area swabs of two revolvers and two semi-automatic pistols were compared to an overall swabbing strategy. The areas of focus for both types of hand guns were the backstrap, grip, trigger, and front sight blade. Samples were also collected from the revolvers' cylinder and hammer and the semi-automatic pistols' slide and magazine.

Standard methods of DNA analysis utilized in forensic laboratories were used, including quantification and amplification. All cleaned firearms were checked for background DNA using control swabbings prior to each handling cycle. Each collection area was first assessed by the average recovered yield of DNA. DNA profiles were then generated and analyzed by comparing the sample profiles to known profiles obtained from the firearms' handlers. Profiles were assessed by examining the total number of alleles out of the possible 30 and total number of complete loci out of 15. Profiles with less than ten loci present were processed with the MinElute® post-purification kit to improve the profile results. Each DNA profile was also checked for allele drop-in, indicating low-level mixtures or contamination.

The focus of this research was to determine whether an overall gun swabbing strategy or an individual area swabbing strategy would produce better DNA profile results. The goal was to discern which one was better in an effort to decrease DNA backlogs by eliminating the large number of samples submitted per case. Upon analyzing all data, it was found that the most efficient collection method was to swab an individual location as opposed to taking an overall gun swab. Both the overall and individual area swabs were capable of producing full profiles; however, the overall gun swabs had a greater chance for allele drop-in and mixtures with a minor contributor when their electropherograms were examined. For the revolvers, the best collection location (average DNA yields >0.01ng/µL, least allele/locus drop-out) was the grip. The semi-automatic pistols' best locations for collection (average DNA yields >0.01ng/µL, least allele/locus drop-out) were the grip and front sight blade. Collections from the backstrap area of both types of hand guns also often yielded substantial quantities of DNA. The magazines of the semi-automatic pistols were good collection areas as well. The triggers of each of the firearms were generally poor targets for swabbing, with low average DNA yields (average DNA yields <0.01ng/µL).

DNA, Firearms, Collection

B14 Secondary or Tertiary Transfer Semen DNA Stains?

Ka-Man Pun, Polizia Cantonale - Scientifica, via Chicherio 20, Bellinzona, Ticino 6500, SWITZERLAND; Giuliana Grimoldi, MSc, Polizia Cantonale - Scientifica, via Chicherio 20, Bellinzona 6500, SWITZERLAND; Gianfranco Foglia, via ferriere, Giubiasco, SWITZERLAND; Ilaria Monico, MS, Police Canton Ticino - Forensic Science Unit, via Chicherio 20, Bellinzona 6500, SWITZERLAND; and Emilio Scossa Baggi, via ferriere, Giubiasco, SWITZERLAND*

After attending this presentation, attendees will better understand: (1) interpreting DNA evidence; (2) the study of transfer and persistence parameters according to a suspect's declarations; and, (3) the evaluation of potential tertiary transfer.

This presentation will impact the forensic science community by the power of accurately interpreting DNA evidence in solving criminal cases.

In sexual assault cases, semen stains can often be found on bodies and/or clothing. The goal of this presentation is to show how an accurate interpretation of DNA profiles can assist investigators in solving a rape case.

The case involved a young victim (V) and two male assailants (A1 and A2). Two men met a drunken woman in a park and engaged in sexual vaginal activity with her. Some hours later, the woman woke up semi-nude in an unknown apartment and called the police. No washing actions (i.e., a shower) were taken between the incident and the collection of the forensic samples from their bodies.

Both men admitted having only one act of sexual intercourse with the woman in the park (first A1 and then A2), but said she consented to the act. Since the woman fell asleep abruptly and they did not know her address, they decided to drive to their apartment. They had no intention of having other sexual activity with the woman; they only wished to help her.

Genetic analysis generated several DNA profiles. By comparison to the reference profiles of A1, A2, and V, the source of the alleles detected in the evidence samples was established. Due to the rough nature of the incident, the victim was bleeding. Several bloodstains mixed with the assailants' semen were found on their clothing and inside the car.

DNA analysis and blood and semen testing indicated the following: (1) vaginal samples contained mixed DNA profiles from V, A1, and A2 (semen and blood positive results); (2) penis and testicle samples from A1 contained mixed DNA profiles from V, A1, and A2 (semen and blood positive results); (3) penis and testicle samples from A2 contained single DNA profiles from A2 (semen positive results).

Given the mixed DNA profiles detected from A1 and considering the suspects' denial of any homosexual relationship between them, the final forensic report discussed the possibility of a secondary transfer of A2 semen following this schema: A2 penis1 victim vagina1 A1 penis. A1 denied having a second act of sexual intercourse with the victim, which would explain the presence of A2's semen on his penis. Rather, A1 described digitally penetrating the victim's vagina inside the car. After doing this, he probably touched his penis, thereby contaminating it with DNA from A2. This kind of scenario involved a tertiary transfer: A2 penis1 victim vagina1 A1 fingers1 A1 penis.

Therefore, A1's fingernail swabs were analyzed and found to have mixed DNA profiles composed of the victim's blood and his own DNA. A2's DNA was not present. When these genetic findings did not support his tertiary transfer hypothesis, A1 tried to justify once again this absence of evidence by a simple hand washing. Consequently, the attention focused on the persistence of semen under the fingernails after an act of digital penetration. It was known that hand washing could have a significant effect on the persistence of trace evidence (trace DNA, fibers, gunshot residues, etc.), but what about the semen? Early forensic papers studied persistence of DNA from laundered semen stains showing the robustness of this kind of biological fluid. Despite this lack of published research in this area, it was possible to assess the evidentiary value of these findings by considering the DNA concentration in spermatic and hematic fluids. In an equal volume, there is more DNA in semen than blood. Therefore, it is very difficult or impossible that a simple hand washing would eliminate the semen while leaving the blood.

Semen DNA Stains, Interpretation, Indirect Transfer

B15 Differentiation of Commercial Ammunition Sources of Unburned and Corresponding Burned Smokeless Powders Based on Chemical Composition Using Mass Spectrometry (MS) and Principal Component Analysis (PCA)

Kristen L. Reese, BA, 5228 Madison Avenue, Apt C7, Okemos, MI 48864; A. Daniel Jones, PhD, 219 Biochemistry, East Lansing, MI 48824; and Ruth Waddell Smith, PhD, Michigan State University, School of Criminal Justice, 560 Baker Hall, East Lansing, MI 48824*

After attending this presentation, attendees will understand the chemical composition differences between unburned and corresponding burned smokeless powders. Attendees will learn how PCA can distinguish commercial ammunitions based on chemical composition.

This presentation will impact the forensic science community by demonstrating the effects of burning on the chemical composition of smokeless powders and the potential of PCA for associating and differentiating powders based on differences in chemical composition.

Smokeless powders are the propellant in firearms. Different organic additives are added to explosive materials to enhance powder performance. Examples are stabilizers including Diphenylamine (DPA) and Ethyl Centralite (EC) and plasticizers including Dibutyl Phthalate (DBP) and Dinitrotoluene (DNT). These organic additives can be analyzed by gas or liquid chromatography coupled to MS. Previous research has shown that differences in abundances of organic additives can distinguish ammunition brands. Masses of fragment ions aid in constituent identification, but targeted Tandem Mass Spectrometry (MS/MS) methods are usually limited by user-defined precursor ions which may preclude detection of novel or unanticipated compounds.

Previous studies investigated changes in powder composition before and after firing. Quantities of Volatile Organic Compounds (VOCs) were analyzed from spent cartridges using headspace extraction methods. Samples were collected at various post-firing time intervals, and VOCs were identified or quantified. No correlation was observed between the quantities of unburned organic additives and VOCs. PCA was only performed on VOCs from fired cartridges, not on corresponding unburned organic compounds. The uncombusted residue within cartridges provides a potential source of organics. As typical collection materials, including swabs, present matrix interferences, a direct solvent extraction from cartridges was reported; however, no comparison to the unburned counterpart was described.

The objectives in this research were two-fold: (1) to investigate differences in organic additives present in a variety of powders, including those more than 15 years old; and, (2) to investigate the composition changes that occur post-firing. A variety of ammunition types and calibers were collected. Five cartridges from each box were selected and unburned powders removed from each. Acetone and dichloromethane were used to extract the organic additives for analysis. Five cartridges from each ammunition type were also fired and collected. A direct solvent extraction was used to recover the burned residue from each cartridge.

All extracts were analyzed by liquid chromatography-high resolution MS. Separated compounds were ionized using Atmospheric Chemical Ionization (APCI) in positive and negative mode with multiplexed non-mass selective Collision-Induced Dissociation (multiplexed-CID) coupled with a time-of-flight mass analyzer for accurate mass data. CID was performed at five collision energies, ranging from 10eV to 80eV, applied between the mass spectrometer ion source and mass analyzer. At each collision energy, fragmentation occurs to different extents. Molecular ions at low collision voltages provide information about the molecular mass of the intact compound. Fragment ions, increasingly prominent at higher collision energies, provide structural information. Structural determination was supported by comparison to relevant standards or published spectra, when available. This non-targeted, comprehensive approach has potential for structural determination of unknown compounds with no available standards.

Chromatograms of each extract were pretreated via background subtraction, retention time alignment, normalization, and scaling. Pretreatment was necessary to minimize contributions of non-sample signals on subsequent data analysis. Using PCA, successful association and discrimination of unburned powders according to the original ammunition was achieved. Notably, powders from different commercial ammunitions contained similar chemical compositions, suggesting the same origin. The first two Principal Components (PC1 and PC2, respectively) accounted for 50% of the data set variance in the data set. Powders from the same ammunition closely clustered, while powders from different ammunition separated in the scores plots. PC1 and PC2 differentiated powders based on differences in abundances of EC and akardite II, respectively. Further differentiation was possible with subsequent PCs influenced by DBP, DPA, and nitroso-DPA.

Chromatograms of burned and unburned powder extracts were compared to assess chemical composition differences. The organic additives in burned powders had reduced abundance, with a depletion of DPA greater than the other additives present. The first three PCs accounted for 47% of the variance, with EC, DPA, and akardite II being most influential. Powders from the same ammunition did not associate as closely due to irreproducibility in the burning process. Powders with considerable chemical differences could be sufficiently discriminated.

Smokeless Powders, LC/MS, PCA

B16 *In Vitro* Experiments Using Human Cadaver Head Hairs to Investigate the Formation Mechanism of Postmortem Hair Root Bands (PMRBs)

Jamie N. Fleming, BS, FBI Laboratory, 2501 Investigation Parkway, Quantico, VA 22135; Hilda S. Castillo, PhD, 4401 Roland Avenue, Unit 107, Baltimore, MD 21210; Ernest J. Drummond, MS, 1220 Blair Mill Road, Apt 209, Silver Spring, MD 20910; Rabih Jabbour, US Army Edgewood Chemical Biological Center, APG, MD 21010; Samir Deshpande, 111 Bata Boulevard, Ste C, Belcamp, MD 21017; Dawnie W. Steadman, PhD, University of Tennessee, Dept of Anthropology, 250 S Stadium Hall, Knoxville, TN 37996; Lee Meadows Jantz, PhD, University of Tennessee, Dept of Anthropology, 250 S Stadium Hall, Knoxville, TN 37996-0720; Kathleen Hauther, University of Tennessee, 250 S Stadium Hall, Knoxville, TN 37920; Jack Hietpas, PhD, FBI-ORISE, 2501 Investigation Parkway, Quantico, VA 22135; Stephen D. Shaw, MS, FBI Laboratory, 2501 Investigation Parkway, Quantico, VA 22135; JoAnn Buscaglia, PhD, FBI Laboratory, CFSRU, 2501 Investigation Parkway, Quantico, VA 22135; Brian Eckenrode, PhD, 2501 Investigation Parkway, Quantico, VA 22135; and Joseph Donfack, PhD, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, attendees will have a better understanding of the biochemical process of hair decomposition and the formation of PMRBs. PMRB is one form of decompositional change presenting as a banded area observed at the proximal end of the root of anagen and early catagen-phase hairs derived from cadavers.[1-5] It has been demonstrated that these banded areas are gas pockets, as they appear dark in transmitted light microscopy and bright in reflected light microscopy.[1] Although the microscopic characteristics of PMRBs have previously been well investigated, the mechanism for their formation requires further research.[1-5]

This presentation will impact the forensic science community by contributing to the understanding of the potential biochemical mechanism(s) of PMRB formation, the chemical species that may be involved, and the protein composition of both banded and non-banded hairs.

Anagen-phase human head hairs were collected from deceased donors of known postmortem interval at the University of Tennessee, Knoxville Anthropology Research Facility. To determine the conditions under which decomposition was promoted or inhibited, non-banded postmortem hairs were trimmed to approximately 1cm in length from the proximal end and immersed in various solutions for 24 days. A visual qualitative assessment of decomposition was performed for each hair at 100x magnification using transmitted light microscopy, before and after immersion. A hair with no visible signs of decomposition was defined as stage 0 in accordance with Koch et al.; stages 1 and 2 refer to slight and full PMRBs, respectively.4

The results from this study support previous findings, indicating that some characteristics of decomposition can be produced when antemortem anagen-phase hairs are submitted to a variety of controlled and uncontrolled (*in vitro*) environmental conditions.[5-10] Of particular note, an ammonium acetate solution (100mM, pH7.0) was found to accelerate the decomposition process of postmortem anagen head hairs (stage 2), and a sodium azide solution (100mM, pH7.5) and protease inhibitor cocktail (3X, pH3.0) exhibited a suppressing effect on hair decomposition (stages 0 and 1), possibly by inhibiting bacterial growth and slowing down protease activities, respectively.[5]

In addition, the protein composition of banded and non-banded postmortem hairs was characterized using liquid chromatography/tandem mass spectrometry. Preliminary qualitative analysis of protein profiles derived from a pool of 20 hairs with stage 2 bands and 20 hairs without bands reveal that fewer proteins are identified in the banded hairs compared to the non-banded hairs. As expected, the majority of the proteins identified are keratin proteins and keratin-associated proteins. It is worth highlighting that no peptidases were identified among the proteins present in the non-banded hair sample group, unlike the banded hair sample group. Overall, these results suggest that peptidases may play a role in PMRB formation through the digestion of proteins and may result in the production of gases that become entrapped between the hair macrofibers.

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Forensic, Hair Decomposition, Postmortem Hair Root Bands

B17 Forensic Soil Analysis by Morphologically Directed Raman Spectroscopy (MDRS)

Andrew C. Koutrakos, MS, 29 Murray Avenue, Shelton, CT 06484; and Brooke W. Kammrath, PhD*, University of New Haven, Forensic Science Dept, 300 Boston Post Road, West Haven, CT 06516*

After attending this presentation, attendees will understand the advantages of using MDRS in the identification of the mineral components present in recovered soil samples. In addition, attendees will better understand how MDRS works and the benefits it can provide the forensic community.

This presentation will impact the forensic science community by demonstrating how MDRS can be used to obtain particle distribution and shape information as well as chemical identifications of minerals in soil samples in order to provide an objective and robust method for their comparison and characterization.

Forensic soil examination is often considered to be very complicated because of the complexity of soil, but such diversity and complexity can be useful as it allows for the differentiation of soil samples with high discriminating power. The complex nature of soil minerals provides a means for its characterization, classification, and comparison.

MDRS combines automated particle imaging and Raman spectroscopy in one instrument. Particle imaging is performed to determine particle size and shape distributions of components in a mixture. Raman spectroscopy is useful for determining molecular chemistry because it is rapid, reliable, does not require contact with the sample, and is non-destructive. Combining these two analytical techniques allows the individual components present within a mixture to be independently characterized and compared. Such a tool can be used to gain a better understanding of mixtures across many areas of forensic science, as it is applicable to a range of Raman active samples. This presentation demonstrates the application of MDRS to soil evidence.

The benefits of MDRS for forensic soil analysis are that not only are you able to non-destructively identify the types of mineral specimens in the soil sample by Raman spectroscopy, but you are also able to obtain morphological information about the individual mineral grains, particle size distributions for the entire sample as well as each of the minerals, and quantitative information on the relative number of each of the particles.

In this research, soil samples were collected from four sites along one road in Connecticut and mineral portions were separated by sieving and washing. The results showed that the morphologies of the mineral fractions were the same, as expected since they were collected along the same road. Prior research has shown that the morphology of quartz can be used to differentiate different mineral environments, which could be exploited with MDRS. Additionally, the particle size distributions of some minerals showed significant differences that can be used to distinguish between samples. Furthermore, the particle counts for each mineral were used for a quantitative comparison between soil samples. This revealed differences between the soil samples. Principal Component Analysis (PCA) was used for exploratory analysis to reveal patterns in the data. In three PCs, good separation was achieved between the four data sets, thus indicating the mineral counts achieved by MDRS can be used for sample discrimination.

In conclusion, MDRS has the potential to be a valuable tool for forensic soil analysis because it is a non-destructive, relatively fast, and automated way to collect particle morphology and chemical information.

Soil Analysis, Morphologically Directed Raman, Raman Spectroscopy

B18 Postmortem Identification From Physiological Biometrics: A Study of Fingerprints, Irises, and Facial Images

Tiffany B. Saul, MS, University of Tennessee, Dept of Anthropology, 250 S Stadium Hall, Knoxville, TN 37996; Kelly Sauerwein, MA*, University of Tennessee, Dept of Anthropology, 250 S Stadium Hall, Knoxville, TN 37996; Dawnie W. Steadman, PhD, University of Tennessee, Dept of Anthropology, 250 S Stadium Hall, Knoxville, TN 37996; and Chris Boehnen, PhD, Oak Ridge National Laboratory, PO Box 2008 MS-6075, Oak Ridge, TN 37831*

After attending this presentation, attendees will understand the use of postmortem fingerprint, iris, and facial biometrics for positive identification of unknown individuals, including the maximum number of days each biometric type can be successfully used for identification and how the decomposition processes of human remains affect the utility of biometrics over time.

This presentation will impact the forensic science community by describing the advantages and limitations of these biometrics based upon the points of similarity and match scores for each identifier type.

Biometrics are measurable unique characteristics that are used to classify both living and deceased individuals. This study examines the effects of decomposition on the ability to correctly identify, or match, individuals over time using three physiological characteristics: fingerprints, facial photographs, and iris scans. This study examines the maximum number of days in which usable biometric data can be successfully matched to an individual using digital technologies and how the image quality and ability to obtain sufficient match scores to make a positive identification decreases over time. For the purposes of this study, *usable data* refers to images that are able to correctly identify the individual through a digital biometric program that uses statistical algorithms to match the captured images with those images taken on the initial receipt of the donated individual. This study was conducted in conjunction with Oak Ridge National Laboratory and the University of Tennessee Anthropological Research Facility between April 2014 and January 2015. Digital facial photographs (n=172), iris scans (n=123), and fingerprints (n=480) from the donated remains of thirteen (n=13) individuals were obtained daily until usable data could no longer be captured. The individuals were placed supine and mostly uncovered with the exception of wire or plastic mesh placed over the hands to prevent scavenger activity. The left iris of all individuals was hydrated with 0.4mL of sterile saline solution ten minutes prior to iris scanning to determine if this would increase the quality of images compared to the untreated right iris. No other preparations were made to the remains prior to data collection.

With daily high temperatures ranging between 59°F (15°C) and 84°F (28.89°C) during the spring trial (n=4), usable data was obtained for an average of four days; however, the early summer trial (n=5) included high temperatures between 81°F (27.22°C) and 91°F (32.77°C) and the number of days usable data could be captured was reduced to two. The winter trial (n=4) only had high temperatures between 18°F (-7.78°C) and 55°F (12.78°C) and demonstrated that useable data was available, on average, for 28 days with both facial images and fingerprints persisting over the longest period at 40.75 days and 24.5 days on average, respectively. Overall, fingerprints produced the most reliable biometric data, with more usable data and higher match scores over longer periods of time than iris scans or facial images. Color change, structural changes to facial features due to bloating, and insect activity prohibited the capture of usable facial images after an average of two days in the spring and summer, while dehydration, clouding, and collapse of the cornea prevented capture of usable iris scans after an average of two days in the spring and only one day in the summer. While the winter trial produced more usable images and significantly higher match scores over a longer period of time, scavenging and freezing temperatures limited the quality and quantity of available data.

This study demonstrated that digitally captured fingerprint biometric data can be used up to 25 days postmortem to identify individuals. The results of this study show that biometrics do remain viable over time, depending upon seasonality and environmental conditions. When scavenger activity is inhibited, fingerprints persist longer than facial and iris identifiers; however, temperature, precipitation, and insect activity were the primary factors affecting the retention of biometric information in decomposing human remains. This study builds upon previous work and continues to support the utility of physiological biometric identifiers during the decomposition process. Postmortem biometric research has the potential to make important contributions to forensic anthropology and the law enforcement, military, and medicolegal communities.

Biometrics, Human Decomposition, Positive Identification

B19 Stability Study of Heroin in Four Common Solvents

Melanie A. Schade, 443 Daniel Street, Allentown, PA 18104; and Thomas A. Brettell, PhD, Cedar Crest College, 100 College Drive, Allentown, PA 18104*

After attending this presentation, attendees will have a better understanding of the stability of heroin in common solvents.

This presentation will impact the forensic science community by providing information about the stability of heroin in common solvents and recommending the best storage conditions for heroin samples and standards in these solvents.

The stability of heroin in four different solvents (acetonitrile, chloroform, methanol, and a 1:9 mixture of methanol:chloroform) was studied. The degradation of heroin in the different solvents under various storage conditions was monitored for 13 weeks. Samples of heroin (100ug/mL) in the four solvents were stored at different conditions, including at room temperature (25°C, +/-3°C), in the refrigerator (7°C, +/-3.0°C), in the freezer (-8°C, +/-3.0°C), and on the autosampler of a gas chromatograph (26°C, +/-3°C). The samples were analyzed in triplicate over a 13-week period using Gas Chromatography/Mass Spectrometry (GC/MS). GC/MS was performed using an Agilent® 7890B gas chromatograph interfaced with an Agilent® 5877A mass spectrometer and an Agilent® 7693 autosampler. The column used was a 30m x 0.25mm x 0.25µm HP-5MS UI. Helium was used as the carrier gas with a flow of 1.0mL/min and linear gas velocity set at 38cm/sec. The inlet, detector, and auxiliary temperatures were 250ºC. The mass scan range was set at 40m/z-500m/z for all samples.

Methanol experiments were repeated and confirmed by GC/MS on a Hewlett Packard 5890 gas chromatograph, GC/MS Agilent® Technologies 6890N Network GC System/5973 Network Mass Selective Detector. The column used was a 30m x 0.25mm x 0.25µm Rxi®5Sil MS. The helium carrier gas flow and linear velocity, temperature settings for column inlet, detector, and auxiliary as well as the mass range were the same as above. The column temperature program was optimized for both columns to ensure baseline resolution of heroin and 6-Monoacetylmorphine (6-MAM). The initial column temperature was 130ºC with a hold time of 2.0 minutes, then the oven temperature was increased @15ºC/min to 250ºC without a hold time and increased again @15ºC/min to 320ºC with a final hold time of 3.0 minutes. 1-µL injections of samples were performed in split mode and with a 50:1 split ratio.

Relative concentration ratios of the degradation products to the heroin concentrations were measured semi-quantitatively using the ratio of area counts of the respective chromatographic peaks. Degradation products were identified through spectral analysis and comparison to known certified standards. It was found that heroin breaks down into 6-MAM in methanol. No breakdown products were observed in acetonitrile, chloroform, or the 1:9 mixture of methanol:chloroform at any of the storage conditions. The percent concentration of heroin in methanol decreased as the temperature of the storage condition increased. For the most part, no breakdown was observed for the heroin in methanol stored in the freezer (-8°C, +/-3.0°C). The methanolic samples stored on the autosampler (26°C, +/-3°C) and at room temperature (25°C, +/-3°C) had the greatest decomposition rate with the lowest percent heroin concentrations and respective highest 6-MAM concentrations. The samples stored in the refrigerator (7°C, +/-3.0°C) had higher percent heroin concentrations than the samples stored at room temperature and on the autosampler of the gas chromatograph. Heroin samples stored in methanol at room temperature were degraded by 10% within a few days and totally degraded in 12 weeks. Based on these results, heroin should not be stored in methanol.

Heroin, Diacetylmorphine, GC/MS

B20 The Utility of Ultra High-Performance Supercritical Fluid Chromatography (UHPSFC) for the Chiral Analysis of Seized Drugs

Stephanie R. Breitenbach, BS, 165 Cross Point Drive, Owings, MD 20736; and Ira S. Lurie, PhD, George Washington University, Dept of Forensic Science, 2100 Foxhall Road, NW, Somers Hall, Lower Level, Washington, DC 20007*

After attending this presentation, attendees will understand the use of UHPSFC for chiral separations. This presentation will also explore the chiral mechanism for UHPSFC.

This presentation will impact the forensic science community by presenting separation conditions for the Supercritical Fluid Chromatography (SFC) chiral separation of certain controlled and non-controlled substances; this can help determine the difference between controlled versus non-controlled drugs, as well as impact trial sentencing and law enforcement intelligence.

The recently introduced separation technique UHPSFC produces highly efficient and rapid separations performed on a new generation of analytical SFC instruments with an environmentally friendly mobile phase, containing as the major component carbon dioxide. Carbon dioxide in the supercritical and subcritical state has properties that are intermediate between a liquid and a gas, giving it excellent diffusivity while maintaining liquid-like properties. UHPSFC, like high-performance liquid chromatography and ultra high-performance liquid chromatography, is advantageous for drugs that are thermally labile, polar and non-volatile, solutes that are problematic for Gas Chromatography (GC) analysis. UHPSFC offers increased selectivity for very similar compounds, such as enantiomers; due to interactions with the stationary phase such as hydrogen bonding, dipole and pi-pi interactions, and a steric fit into a chiral surface. In this vein, UHPSFC is amenable to the chiral separation of drugs of forensic interest.

This project will highlight the use of three chiral columns, the AMY1, CEL1, and CEL2 which contain amylose and cellulose respectively as the chiral backbone. These columns were studied for the chiral separation of synthetic cannabinoids, bath salts, and phenethylamines, including methamphetamine. The studies were performed using carbon dioxide (CO2) with several different modifiers and additives. The modifiers include methanol, acetonitrile, ethanol, and isopropanol, while the additives include ammonium formate and ammonia. The synthetic cannabinoids studied consisted of four controlled drugs, including CP 47, 497, its diastereomer epi-CP 47, 497 and their C8 homologues, as well as two non-controlled positional isomers of controlled JWH-018. The "bath salts" investigated included 14 controlled drugs, in addition to seven non-controlled positional isomers of controlled pentedrone and 4-methylcathinone, four non-controlled positional isomers of controlled mephedrone and buphedrone, three non-controlled positional isomers of controlled α-PVP, two non-controlled positional isomers of controlled 4-MEPP and α-PBP, one non-controlled positional isomer for controlled methcathinone, methylone, butylone, pentylone, and MDPV, respectively. The phenethylamines studied included amphetamine, methamphetamine, MDA, MDMA, and MDEA.

The use of UHPSFC enabled the baseline separation of all of the studied enantiomers of synthetic cannabinoids, and dl-methamphetamine. UHPSFC resolved 9 out of 14 enantiomers of the controlled "bath salts", all 21 of the non-controlled positional isomers of the latter solutes, and MDMA with a resolution of 1 or better.

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Chiral, Seized Drugs, Supercritical Fluid

B21 Analysis of Black Electrical Tapes by Direct Thermal Extraction-Gas Chromatography/Mass Spectrometry (TE-GC/MS)

Emily Prisaznik, BS, Cedar Crest College, 100 College Drive, Allentown, PA 18104; and Thomas A. Brettell, PhD, Cedar Crest College, 100 College Drive, Allentown, PA 18104*

After attending this presentation, attendees will have a better understanding of how TE-GC/MS can be used to analyze volatiles thermally extracted from electrical tapes.

This presentation will impact the forensic science community by providing an alternative method to analyze and compare electrical tapes that may be submitted as trace evidence to crime laboratories.

Black Polyvinyl Chloride (PVC) electrical tape is often used in the construction of Improvised Explosive Devices (IEDs) as a means of securing their components. Consequently, black electrical tape is often submitted as evidence to crime laboratories. IEDs often incorporate PVC tape for sealing, insulating, or securing parts to the device; therefore, important information can be gained from the analysis of the components of IEDs, either intact or fragmented. Most of the methods that have been developed for the forensic analysis of black electrical tape have used a combination of microscopy, Infrared (IR) and/or Raman spectroscopy, Scanning Electron Microscopy/Energy Dispersive Spectroscopy (SEM/EDS), or Pyrolysis Gas Chromatography (PyGC). New alternative methods to compare and differentiate black electrical tapes are needed.

For this reason, Direct Thermal Extraction-Gas Chromatography/Mass Spectrometry (DTE-GC/MS) was explored as an analysis technique for black electrical tapes. Thermal sampling techniques generally require no sample preparation and can replace more complex and time consuming analytical procedures such as solvent extraction. DTE is a thermal desorption method used to extract Volatile Organic Compounds (VOCs) and semivolatile compounds directly to the gas chromatographic column for analysis. DTE-GC/MS can differentiate and identify volatiles thermally extracted off of polymers such as PVC tapes. This thermal extraction technique is a dynamic process that can be used to analyze both solid and liquid samples. Under a continuous flow of inert gas and heat, volatile and semi-volatile organics are thermally extracted from the sample matrix into the gas stream and transferred to the vapor phase and then into the carrier gas of a gas chromatograph. DTE-GC/MS was used in this experiment to examine black electrical PVC tape samples purchased from various commercial sources. DTE was performed using an SIS AutoDesorb™ system. The AutoDesorb™ tower, containing the sample analysis hardware, sits over the gas chromatographic injection port and communicates with the PC software through the electronics console. Trace amounts (<30mg) of samples were thermally desorbed at 150°C with a purge and flow of helium gas for one minute. Desorption time for all samples was set to ten minutes with an initial column trap temperature at 30°C to focus the volatiles onto the gas chromatographic column.

GC was performed using a 5890 Agilent® gas chromatogaph. A Zebron™ ZB-5HT capillary column (30m+5m Guardian x .25mm i.d. x .10µm) was used for all analyses. A helium carrier gas with a flow rate of 1.0mL/min was used with a 10:1 split. The injector temperature was set to 225°C. The initial column temperature was held at 30°C for two minutes, followed by its first ramp to 150°C increasing 10°C/minute. The second ramp was to 240°C increasing 2°C/minute, finishing with a final ramp to 280°C increasing 10°C/minute. The oven was held at 280°C for five minutes. A post-run program returned the oven temperature to 30°C and lasted ten minutes. MS was carried out on a 5973 Agilent® Mass Selective Detector with a scan range of 40m/z-500m/z and auxiliary temperature of 250°C.

All the black electrical tapes studied produced different chromatograms with peaks of varied intensity. All samples were run in triplicate with excellent reproducibility. The compounds identified originated from volatiles thermally extracted from the tape film and adhesive. Phthalate plasticizers from the tape film were among these peaks. In conclusion, this study provides an innovative and semi-destructive technique to examine trace amounts of polymeric materials, specifically PVC electrical tape that may be submitted to crime laboratories.

Electrical Tape, Direct Thermal Extraction, GC/MS

B22 Think Outside the Box: External Human Factors on the Analysis, Comparison, Evaluation-Verification (ACE-V) Methodology

Francisco Valente Gonçalves, MSc, University of Leicester, Dept of Criminology, 154 Upper New Walk, Leicester LE1 7QA, UNITED KINGDOM; Lisa L. Smith, PhD, University of Leicester, 154 Upper New Walk, Leicester LE1 7QA, UNITED KINGDOM; and Doug Barrett, PhD, University of Leicester, Henry Welcome Bldg, School of Psychology, Leicester, UNITED KINGDOM*

After attending this presentation, attendees will gain a different perspective on the topic of cognitive bias within forensic sciences, namely fingerprint examinations. The goal of this presentation is to suggest some external variables which have not been researched to date and which can contribute to the increase or decrease of latent print examiners' performance during their workflow.

This presentation will impact the forensic science community by providing a different way to observe cognitive contaminations by focusing on external variables such as feedback, accreditation, opportunities for funding, and external pressures from other professionals. In this way, this study looks for the responsibility of other professional roles within the legal system (managers, external consultants, lawyers, and judges) in regard to the topic of miscarriages of justice.

In 2004, in the Madrid bombing case, Federal Bureau of Investigation (FBI) latent print examiners identified Brandon Mayfield as the contributor of a fingerprint associated with the terrorist attack due to a misguided decision within the fingerprint analysis. Research conducted on human factors since that event showed that one possible explanation for the error made by the latent print experts was contextual bias; however, this was not specific to only this case within a forensics laboratory in the United States nor even specific to a particular forensic discipline.

In recent years, the forensic community has found, in a diversity of cases, various flaws in their procedures which have led to miscarriages of justice within the legal system and have required laboratories to reanalyze cases where errors may have occurred.

Due to these incidents, the academic community has contributed research providing governmental institutions with empirical data on human factors and contributing to official reports regarding quality procedures to circumvent issues such as cognitive contaminations.

It has been noted that forensic laboratories around the world have now started to embrace quality and accreditation standards. There is an increased need to seek high levels of quality within the procedures that practitioners undertake in the various disciplines of forensic science during their everyday work flow.

Although there is already research on human factors, this presentation suggests that the majority of the observed variables are associated with what might be called "internal variables," since these are concerned with factors regarding personal characteristics of the latent print examiners (e.g., fatigue, stress, stereotypes, and cognitive contaminations in general).

Thus this study was interested in discussing the plausibility of looking for "external variables" such as feedback from managers, accreditation agencies, and external consultants which can lead to overconfidence, opportunities for funding which enables the laboratory to improve its methodology, equipment, and human resources, and also external pressures from investigators and other departments of investigation, lawyers, or even judges which can lead to contextual bias. These external pressures are believed to be the source of increasing or decreasing motivation within examiners' work flows which affects their performance.

By investigating the impact these variables have on forensic practitioners' performances, other roles of the legal system will be included in this discussion as well as how to achieve a more holistic understanding of the causes of error and how to manage these risks to avoid future miscarriages of justice.

With strong collaborations between different institutions (e.g., forensic laboratories, departments of investigations, courts, and accreditation agencies), it is possible to improve quality assurance and quality control for forensic procedures, thus improving the legal system overall.

Fingerprints, Errors, Standards

B23 Liquid Chromatography/Mass Spectrometry (LC/MS) Method Development for the Identification of Route-Specific 3,4-Methylenedioxymethamphetamine (MDMA) Impurities

Rebecca F. Dunn, 885 N Easton Road, Apt 3A6, Glenside, PA 19038; and Heather L. Harris, MFS, JD, PO Box 43626, Philadelphia, PA 19106*

After attending this presentation, attendees will better understand the benefits of using LC/MS instrumentation over Gas Chromatography/Mass Spectrometry (GC/MS) for impurity profiling of amphetamine impurities.

This presentation will impact the forensic science community by demonstrating the simple extraction techniques possible with LC/MS instrumentation.

MDMA is a Schedule 1 psychoactive hallucinogenic stimulant commonly found in "Ecstasy" tablets or referred to as "Molly." By analyzing the organic by-products, or impurities, in MDMA tablets, it is possible to identify the synthetic route used to prepare the sample. Two of the most common methods of synthesis are the reductive amination of MDP-2-P and the Leuckart reaction. The differentiation between these two synthetic routes can aid investigators in the identification of the manufacturer of the sample and help to compare tablets from multiple drug seizures.

In the past decade, several GC/MS techniques have been developed to analyze MDMA tablets and to identify common route-specific impurities. It is hypothesized that LC/MS will be more suitable for this research due to its increased sensitivity and ability to analyze more polar compounds without derivatization. The goal of this research was to develop a simplified method for the extraction and identification of 11 previously identified route-specific MDMA impurities using dry extraction techniques and LC/MS instrumentation. By only identifying the impurities selected, this method is able to focus on the impurities, typically found at low concentrations, which indicate these two popular synthetic routes. In order to achieve this goal, an LC/MS method was designed to identify the compounds and several extraction methods were evaluated for the optimal extraction of the compounds of interest from simulated tablet matrices.

Of the impurities chosen, eight were indicative of the reductive amination route. These compounds were: N-cyclohexylacetamide; 3,4-methylenedioxyamphetamine (MDA); p-methoxymethamphetamine (PMMA); N-(1,3-benzodioxol-5-yl-methyl)-N-methylamine; 3,4-methylenedioxyacetophenone; 3,4-methylenedioxypropiophenone; methyl piperonylate; and 3,4-methylenedioxyethylamphetamine (MDEA). The remaining three were indicative of the Leuckart reaction. These compounds were: N-ethylamphetamine; N-formylmethamphetamine; and N,1,7,7-tetramethylbicyclo-[2.2.1]-heptan-2-amine. In addition to the impurities that were analyzed, the LC/MS method was designed to identify MDMA and caffeine in order to ensure that the presence of the primary ingredient and a popular additive would not inhibit the identification of the analytes of interest. Benzylmethylamine was used as an internal standard.

The LC/MS method developed for the identification of the analytes of interest was able to qualitatively identify all compounds at concentrations above 2µg/mL and identify all amphetamine analytes at concentrations above 1ng/mL. All analytes were positively ionized and baseline separated.

Three dry extraction methods were utilized for the extraction of the compounds of interest from tablets. The compounds of interest were extracted from four different common excipients: cornstarch; d-lactose; d-sorbitol; and microcrystalline cellulose. The extraction solvents were methanol, 0.05 N hydrochloric acid in methanol, and 0.1% trifluoroacetic acid (TFA) in methanol. Percent area ratios were used to determine the percent recovery of each method and these results were compared to the percent recoveries obtained from a previously optimized liquid-liquid extraction method. When extracted from cornstarch, the liquid-liquid extraction had the highest average percent recovery for all compounds at 58%. Of the three dry extraction methods, the methanolic hydrochloric acid had the highest average percent recovery at 52%, with the methanolic TFA having 48% and the methanol having 42%. When the percent recoveries for the individual analytes are compared, the data shows that the methanolic hydrochloric acid was the optimum extraction method for a majority of the analytes, but lower recoveries for MDMA and caffeine drastically reduced the overall average.

Liquid Chromatography, MDMA, Impurity Profiling

B24 Forensic Analysis of Human Autopsy Tissue for the Presence of Polydimethylsiloxane (Silicone) and Volatile Cyclic Siloxanes Using Macro Fourier Transform Infrared (FTIR) Spectroscopy, Micro-FTIR Spectroscopic Imaging, and Headspace/Gas Chromatography With Mass Spectrometric Detection (HS/GC/MS)

Caroline Machal Kelley, BS, USFDA-FCC, 6751 Steger Drive, Cincinnati, OH 45237; and Adam C. Lanzarotta, PhD, USFDA-FCC, 6751 Steger Drive, Cincinnati, OH 45237*

After attending this presentation, attendees will see the advantages of using HS/GC/MS, in addition to those offered by FTIR spectroscopy, to provide complementary data yielding a strong indication for the presence of silicone in human autopsy tissue.

This presentation will impact the forensic science community by showcasing effective and minimally labor-intensive primary FTIR and secondary HS/GC/MS methods for the detection of silicone in human autopsy tissue.

The United States Food and Drug Administration's Forensic Chemistry Center (FCC) has received multiple requests over the past 15 years to examine hypodermic needles, syringes, and unknown liquids for the presence of polydimethylsiloxane (PDMS) (silicone); however, in the past five years, due to the rise in silicone injection popularity including "pumping parties," the FCC has been tasked with analyzing human tissue samples for PDMS. In these cases, PDMS was allegedly injected into patients' lips, face, breasts, buttocks, and/or other areas of the body for cosmetic enhancement by unlicensed individuals. The practice has led to several serious health complications, including death. In the event of a death, the medical examiner often requests that the victim's autopsy tissues be examined for the presence of PDMS in order to help determine the extent to which the PDMS has migrated in the body, which may ultimately help the medical examiner determine the cause of death.

Benefits of FTIR and Raman methods, both point mode and imaging mode, include the ability to provide PDMS-specific, solid-state (morphological), *in situ* examinations of biological tissue inclusions. The disadvantage is that prior to analysis, these studies required the tissue to be rinsed with an organic solvent, cross-sectioned with a microtome, and carefully mounted to an IR-reflective substrate, which is not typically how autopsy tissue samples are received by the FCC. Furthermore, this approach can be unnecessary and prohibitively time consuming for applications that do not require morphological information via an *in situ*, nondestructive approach (i.e., simply answering the question of whether or not PDMS is present in autopsy tissue). As a result, it was of interest to develop a straightforward primary PDMS-specific method using FTIR spectroscopy and/or FTIR spectroscopic imaging for the analysis of human autopsy tissue received directly from the medical examiner with little or no treatment or preparation.

GC/MS was considered as a secondary technique because of the low detection limit and high selectivity/sensitivity of this method for detecting Volatile Cyclic Siloxanes (VCSs). The drawback to this approach is that the GC/MS method required significant sample preparation and the results may yield a broad peak manifold for silicone. On the other hand, HS/GC/MS, which, as far as this study could determine, has not yet been utilized for the determination of VCS impurities as marker compounds for PDMS in human autopsy tissue, offers several advantages compared to conventional GC/MS methods. First, HS/GC/MS requires less sample preparation because the sample can often be analyzed neat. Even if the sample cannot be examined neat due to low VCS concentrations, minimal sample preparation is required; the tissue sample is extracted with hexane and the extract is analyzed. Second, regardless of whether the tissue sample is examined neat or as an extract, the HS/GC/MS analysis yields a cleaner chromatogram because the broad silicone peak observed using GC/MS is not observed in the HS method since PDMS has a much lower vapor pressure than the VCSs and therefore will remain in the tissue sample or extract liquid.

Effective and minimally labor-intensive primary and secondary methods have been developed for the detection of silicone in human autopsy tissue. The primary method is PDMS-specific and employs either macro Attenuated Total Reflection/Fourier Transform Infrared (ATR/FTIR) spectroscopy (for samples with a relatively high PDMS concentration) or micro FTIR spectroscopic imaging in a reflection/absorption modality (for samples with a relatively low PDMS concentration). Although the secondary method is not PDMS-specific, it is a novel approach and employs HS/GC/MS for the detection of VCSs, which are characteristic marker compounds for PDMS.

These techniques and methods have been successfully employed in 11 cases involving the examination of various types of human tissues for the presence of PDMS. To date, the offenders have been found guilty of felonies including third-degree murder, depraved heart murder, and criminally negligent homicide with convictions ranging from several months to life in prison.

Silicone, Human Autopsy Tissue, HS/GC/MS

B25 Breaking Forensic Boundaries: Developing International Standards

Soraya McClung, Houston Forensic Science Center, 1200 Travis Street, 20th Fl, Houston, TX 77046; and Kermit B. Channell II, BS*, Arkansas State Crime Laboratory, #3 Natural Resources Drive, Little Rock, AR 72205*

The goal of this presentation is to provide information about global initiatives whose purposes are to develop international standards for both forensic science providers and for the manufacturing of forensic products.

This presentation will impact the forensic science community by educating attendees on the purpose and objectives of the International Organization for Standardization Technical Committee (ISO/TC) 272. This presentation will include background information on ISO/TC 272, recent achievements, and future projects.

ISO/TC 272 has been established to develop internationally accepted standards relating to the delivery of forensic science services. Such standards primarily apply to organizations that analyze and/or interpret physical evidence for the purposes of presenting conclusions to a court of law. The standards are designed to preserve the features of evidence subject to observation and to maintain the integrity of that evidence through each stage of testing. They are also designed to facilitate information sharing between international jurisdictions.

The quality and nature of the materials, reagents, and consumables used by forensic service providers during testing can negatively impact the features of the physical evidence under examination. As such, the committee also develops standards directed toward manufacturers. These standards are meant to control the production of such materials to ensure they are fit for forensic purposes. The committee recently produced the Final Draft International Standard (FDIS) 18385, "Minimizing the risk of human DNA contamination in products used to collect and analyze biological material for forensic purposes."

In 2009, the National Academy of Sciences (NAS) Report, Strengthening Forensic Science in the United States – A Path Forward, highlighted the lack of forensic standards and the potential impact this was having on the administration of justice. These criticisms are applicable worldwide. A number of countries are now in the process of developing national forensic standards. Standards Australia has published AS5388 Forensic Analysis. Comité Européen de Normalisation (CEN) is currently developing a draft standard for the collection of forensic evidence. There are also a number of American Society for Testing and Materials (ASTM) standards in existence. The continuation of standards being developed in each country without cross-border coordination and collaboration may limit the exchange of forensic evidence and intelligence across international borders. This lack of communication could also negatively impact the investigation of global crime including terrorism, fraud, and child exploitation. This is also creating duplication of effort by ISO member bodies.

The objectives of the TC are to develop standards that: (1) enhance the reliability of forensic evidence; (2) establish consistent work practices that facilitate forensic laboratories/agencies from different jurisdictions to work collaboratively in response to cross-border investigations; (3) enable agencies from different jurisdictions to support one another in the event of a catastrophic event that exhausts a jurisdiction's capabilities; and, (4) allow for the exchange of forensic results, information, and intelligence, including the sharing of databases.

Standards, International, Technical Committee

B26 Human Scent Evidence — Volatile Organic Compounds (VOCs): A Unique Trace From Science to Criminal Investigation

Marcello Rendine, Viale degli Aviatori 1, Foggia 71100, ITALY; Cristoforo Pomara, MD, PhD, University of Foggia, Dept Forensic Path, University of Malta, Dept of Anatomy, Faculty of Med & Surg Biomedical Sci, Foggia, Misida, Malta 71100, ITALY; Alessandro Bellifemina, Viale degli Aviatori 1, Foggia 71100, ITALY; Dania De Carlo, MD, Ospedale Colonnello D'Avanzo, Viale degli Aviatori 1, Foggia 71100, ITALY; Carmela Fiore, MD, Ospedale Colonnello D'Avanzo, Viale degli Aviatori 1, Foggia 71100, ITALY; Palmira Fortarezza, MS, Ospedale Tatarella, Cerignola, ITALY; Margherita Neri, MD, PhD, University of Foggia, Dept of Forensic Pathology, Viale degli Aviatori 1, Foggia 71100, ITALY; and Irene Riezzo, MD, PhD, University of Foggia, Osp D'Avanzo, Dept of Forensic Pathology, Viale degli Aviatori, 1, Foggia 71100, ITALY*

The goal of this presentation is to demonstrate to the forensic science community the specific VOCs from human scent evidence that elicit an appropriate response by properly trained scent-discriminating canines.

This presentation will impact the forensic science community by explaining that the identification of VOCs released by a unique human scent may be useful aids in training scent-discriminating dogs to use in criminal investigations.

Whenever a crime is committed, forensic personnel are requested in order to collect crime scene evidence to establish relationships between suspects and the crime. In the event that physical evidence is destroyed or not found, there is one type of latent evidence that is always deposited at the crime scene: the unique human scent. Although the concept of human scent as forensic trace evidence is not new, there is currently very little understanding of how human scent is produced and of its uniqueness.

Recent increases in the use of trained canines for selectively discriminating human scent has created the need to have an exact knowledge and awareness of the volatile chemical signature of compounds that could indicate the presence of an alleged offender at the crime scene.

The uniqueness of human scent is based on the combination of many factors including diet (primary odor), environmental influences (secondary odor), external sources (tertiary odor), and genetics. Although human scent is defined by the most abundant VOCs, only a few VOCs can become airborne and detected by the canine olfactory system.

This study was performed using Gas Chromatography/Mass Spectrometry (GC/MS) to identify the VOCs released from humans that stimulate canine olfactive discriminating alerts and the indirect scenting system. Scents in this study were collected from eight people (four male and four female). These subjects washed their hands under running water using the same soap and waited for them to dry naturally without contact with any object or person. The experimental subjects then touched some objects (the scent articles) in the same room in order to imprint their odor.

For the indirect scenting system training, the surface of the scent article was wiped with 40 sterile and VOC-free cotton gauze pads; likewise, for a passive absorption, 40 sterile and VOC-free cotton gauze pads were placed on the scent article for 48 hours. These were stored in 80 separate glass jars covered by a sterile, VOC-free film and closed with a lid. The jars were stored at constant room temperature (22°C and 45% relative humidity). The gauze pads were subsequently presented to the canine to sniff and stimulate discriminating alerts.

The gauzes were used in part for the chemical analysis and in part for the dog training procedure. The first extraction was assessed on the gauze as time 0 of the experiment. The headspace extractions were repeated every 5 days for 20 days, resulting in 40 extractions. The National Institute of Standards and Technology (NIST) mass spectral library and extracted ion chromatograms were used to identify the compounds.

During the analysis, more than 50 VOCs were identified. Only VOCs originating from human specimens were used in the analysis of the samples as key markers of the presence of the suspect at the crime scene. The various molecules were selected and analyzed so as to identify and assess their changes according to the experimental subject's unique human scent.

The results of this study indicate that the professionally trained scent identification dog is an outstanding biological device for collecting invisible trace evidence at the scene of crime, displaying excellent sensitivity (between 99.42% and 100%) and specificity (between 99.05% and 100%) and having a Positive Predictive Value (PPV) ranging between 97.94% and 100%, and a Negative Predictive Value (NPV) ranging between 95.71% and 100%.

Human Scent Evidence, Volatile Organic Compounds, CSI Canines

B27 Chemical and Canine Analysis as Complementary Techniques for the Identification of Active Odors in a Biothreat Agent

Alison Simon, BS, 11200 SW 8th Street, CP304, Miami, FL 33199; Julian L. Mendel, MSc, 10005 SW 141 Court, Miami, FL 33186; Kenneth G. Furton, PhD, Florida International University, International Forensic Research Institute, University Park, Miami, FL 33199; and DeEtta Mills, PhD, Florida International University, OE 167, Biological Sciences, 11200 SW 8th Street, Miami, FL 33199*

After attending this presentation, attendees will better understand the biothreat agent *Raffaelea lauricola*, which causes the lethal laurel wilt disease. Attendees will understand how research is combating the spread of this fungus by using a combination of chemical analysis and canine trials to identify the active odors and create a safer, longer-lasting training aid.

This presentation will impact the forensic science community by introducing a novel method of identifying active odors to be used in the field of forensic canines in order to prevent the spread of *R. lauricola* through canine detection. This presentation will also strengthen the validity of canine detection as it is currently used in forensic science.

Canines have served an integral part of forensic science for more than a century, yet there is little science to support their ability to distinguish Volatile Organic Compounds (VOCs) of illegal or controlled substances. By identifying the odors to which canines alert, it is possible to create safer, longer-lasting training aids, as well as provide scientific support in legal proceedings. In the case of the invasive biothreat agent *R. lauricola*, canines are currently the only method of early detection. *R. lauricola* is a fungus that was brought into the country in the early 2000s and is currently devastating avocado groves in the United States. It causes the laurel wilt disease that kills trees within six weeks. The fungus is carried by an invasive beetle (*Xyleborus glabratus*) which bores into a host tree and farms the phytopathogenic fungus as food. The tree attempts to halt the spread of the fungus by systematically shutting down its respiratory system, which unintentionally stops the spread of nutrients and water and kills the tree. Once a biothreat or other banned agricultural item has entered the country, there is no established, uniform method of eradication.

The current study used Solid Phase Microextraction/Gas Chromatography/Mass Spectrometry (SPME/GC/MS) to identify the odors present in avocado trees infected with the pathogen. Twenty-eight compounds were identified using this method; however, most of these compounds were not commercially available. In order to create a training aid for canines trained to detect *R. lauricola*, the compounds the canines are alerting to had to be identified. To this end, two separate canine trials were completed. First, four canines were run on Controlled Odor Mimic Permeation Systems (COMPS) made of infected tree wood, uninfected tree wood, and fungus cultures. All canines successfully alerted to infected tree wood and fungus cultures, but not uninfected tree wood, proving that the canines are alerting to fungal odors present in infected trees with a positive predictive value of 98.3%. The second trial was designed to identify these odors without the assistance of pure compounds, since they are not commercially available. By venting a GC column to the atmosphere, fractions of the chromatograph were collected. These fractions were presented to the canines in a series of trials, resulting in the identification of a portion of chromatogram that the canines alert to as active odors for the biothreat *R. lauricola*. Using the fraction identified by the canines, an environmentally safe and longer-lasting training aid will be created. Additionally, a new method of odor identification was created for future use in the field of forensic canines.

Biothreat, Canine Detection, *Raffaelea Lauricola*

B28 Investigating the Use of New Psychoactive Substances (NPS) Using Sewage-Based Epidemiology (SBE): Detection and Identification of Transformation Products (TPs) of Methylone and Methylenedioxypyrovalerone in Sewage Using Accurate-Mass Mass Spectrometry (MS)

Juliet Kinyua, MSc, Universiteitsplein 1, Berchem, Antwerp 2600, BELGIUM; Noelia Negreira, PhD, University of Antwerp, Universiteitspein 1, Campus Drie Eiken, Wilrijk 2610, BELGIUM; Ann-Kathrin McCall, MSc, Eawag, Swiss Federal Institute of Aquatic Science, CH-8600 Dübendorf, Zurich, SWITZERLAND; Christoph Ort, PhD, Eawag, Swiss Federal Institute of Aquatic Science, CH-8600 Dübendorf, Zurich, SWITZERLAND; Adrian Covaci, PhD, University of Antwerp, Universiteitsplein 1, Antwerp 2610, BELGIUM; and Alexander van Nuijs, PhD, University of Antwerp, Universiteitspein 1, Campus Drie Eiken, Antwerp 2610, BELGIUM*

After attending this presentation, attendees will understand non-target strategies based on Liquid Chromatography (LC) coupled to quadrupole Time-of-Flight/Mass Spectrometry (qTOF/MS) to identify NPS and their in-sewer TPs which are useful in SBE. This presentation demonstrates the elucidation of tentative structures of TPs of two NPS: methylone and methylenedioxypyrovalerone (MDVP).

This presentation will impact the forensic science community by revealing recent developments in SBE for the detection and identification of NPS use. This presentation illustrates potential NPS biomarkers and provides an approach for their identification in sewage.

Introduction: The SBE approach relies on the analysis of excretion products of Illicit Drugs (IDs) in sewage with the purpose of estimating community drug use. It is based on the fact that an ingested dose of ID is metabolized and excreted mostly in urine as parent compound and metabolites ending up in influent sewage. It uses concentrations of ID and metabolites in influent sewage to back-calculate amounts of these substances used by a community. SBE has been applied since 2005 as an approach complementary to classical investigation methods such as medical records, population surveys, and crime statistics for estimating ID use in communities.

While SBE has been applied repeatedly for the estimation of conventional ID use, only a few studies have quantified NPS in sewage. These studies have shown that NPS concentrations in sewage are generally very low. Also, it would be worthwhile to explore the possibility of other biomarkers since parent compounds may be subject to metabolism and transformation during their in-sewer transport.

In this study, the stability of methylone and MDPV in sewage and the possible formation of TPs were studied in the presence of biofilm. The experiments were conducted individually for each selected compound over 24h.

Methods: The LC system consisted of an Agilent® 1290 Infinity LC and separation was performed using a Phenomenex Biphenyl (100 x 2.1mm, 2.6µm) at a flow rate of 0.4mL/min. The mobile phase consisted of: (1) H2O with 0.04% HCOOH; and, (2) 80/20 AcN/H2O with 0.04% HCOOH. The applied gradient, in function of B, was: 0min, 2%; 2min, 2%; 18min, 40%; 25min, 90%; 29min, 90%; 29.5min, 2%; and 33min, 2%. The MS system consisted of an Agilent® 6530 Accurate-Mass qTOF instrument operated with jet stream electrospray ion source. The source parameters were as follows: gas temperature, 325°C; gas flow, 8L/min; nebulizer gas, 40psi; sheath gas temperature, 325°C; sheath gas flow, 11L/min; and capillary voltage, 3,500V and the nozzle voltage, 0V. The data-independent acquisition (All-ions MS/MS) was set up to acquire three scan segments in MS mode alternating the collision energies 0eV, 15eV, and 35eV, respectively. With this acquisition mode, in only one injection, data are acquired in scan segment one to display the "precursor ion" (0eV), and scan segment two and three to provide the product ions (15eV and 35eV).

Samples collected over the 24h period were first centrifuged at 8,000rpm for seven minutes before directly injecting them into the LC/qTOF/MS system.

Results: Results showed that after 24h, MDPV and methylone were not stable compounds, with only 67% and 59% of the initial concentration remaining, respectively. MDPV transformation in the presence of biofilm revealed the formation of three TPs corresponding to the loss of the methylene group (*m/z* 264.1579, C15H21NO3), di-hydroxylation (*m/z* 308.1467, C16H21NO5), and hydroxylation in the pyrrolidine ring, oxidation and ring opening (*m/z* 292.154 (C16H21NO4).

Methylone transformation also revealed formation of three TPs: loss of water by the formation of a conjugated indole system between the primary amine and the aromatic ring (*m/z* 188.0678 (C11H9NO2); reduction of the group ketone (*m/z* 210.1115, C11H15NO3); and loss of the methylenedioxy moiety after formation of the indole system (*m/z* 146.0587, C9H7NO).

This study successfully utilizes data-independent acquisition and non-target data processing techniques in the identification of TPs from MDPV and methylone. These developments in identification of the most suitable biomarker could improve detection of NPS using the SBE approach and serve as an early warning system to stakeholders.

Non-Target Strategies, Sewage-Based Epidemiology, NPS

B29 Updates From the Drug Enforcement Administration National Forensic Laboratory Information System (NFLIS): Opiates and Related Drugs Reported in NFLIS — 2009-2014

DeMia P. Pressley, MS, Drug Enforcement Administration, Office of Diversion Control, 8701 Morrissette Drive, Springfield, VA 22152; Artisha Polk, MS, Drug Enforcement Administration, Office of Diversion Control, 8701 Morrissette Drive, Springfield, VA 22152; Liqun Wong, MS, 8701 Morrissette Drive, Springfield, VA 22152; Kevin Strom, PhD, 3040 Cornwallis Road, Research Triangle Park, NC 27709; Katherine N. Moore, MS, RTI International, 3040 E Cornwallis RD, RTP, NC 27709; David Heller, BS, RTI International, 3040 E Cornwallis Road, RTP, NC 27709; Jeffrey M. Ancheta, BS, 3040 Cornwallis Road, Research Triangle Park, NC 27709; BeLinda J. Weimer, MA, 3040 Cornwallis Road, Research Triangle Park, NC 27709; Hope Smiley-McDonald, PhD, RTI International, 3040 E Cornwallis Road, Research Triangle Park, NC 27709; and Jeri D. Ropero-Miller, PhD, RTI International, 3040 Cornwallis Road, PO Box 12194, Bldg 7, Rm 211, Research Triangle Park, NC 27709*

After attending this presentation, attendees will understand the breadth of information that the NFLIS provides to the forensic community.

This presentation will impact the forensic science community by providing specific knowledge of national and regional trends for opiates and related substances as reported to NFLIS between 2009 and 2014.

The objective of this presentation is to provide the community with a special report on opiates and related drugs reported to NFLIS from 2009 to 2014 and highlight two NFLIS resources, the Data Query System (DQS), and the Drug Information System.

NFLIS is a program of the Drug Enforcement Administration (DEA), Office of Diversion Control that collects drug identification results from cases analyzed by federal, state, and local laboratories. The system currently includes data from laboratories that conduct analyses of more than 91% of the nation's approximately one million annual state and local drug cases. A total of 278 individual laboratories from state systems and local or municipal laboratories/laboratory systems participate in NFLIS. Results from NFLIS are regularly used to support drug scheduling efforts and to aid drug initiatives, including the identification and tracking of emerging drugs of abuse.

Semiannual national estimates from January 2009 to June 2014 are presented for 16 opiates and related substances, as are reports of fentanyl identified with other drugs in the same item. Maps showing state- and county-level reports of oxymorphone, hydromorphone, and fentanyl are also presented. NFLIS results for emerging opiate-related drugs such as mitragynine, acetyl fentanyl, AH-7921, MT-45, and desomorphine are shown. Federal data from DEA and United States Customs and Border Protection laboratories are presented, along with data from IMS Health's™ National Prescription Audit Plus Retail database, the DEA's Automation of Reports and Consolidated Orders System (ARCOS), and Centers for Disease Control and Prevention (CDC) medical examiner data on deaths associated with opiates and related drugs.

From January 2009 to June 2014, an estimated 1,438,933 opiates and related drugs were reported to NFLIS. The number of reports increased by 28% over this period, from 116,647 drug reports during the first half of 2009 to 149,722 during the first half of 2014. From the first half of 2009 to the first half of 2014, hydromorphone reports more than doubled in the South from a rate of 0.75 reports to 2.05 reports per 100,000 persons (679 to 1,965 reports). All other regions fluctuated between minor increases and decreases in hydromorphone reports within the six-month reporting periods. Fentanyl reports increased by 300% from the second half of 2013 to the first half of 2014. This increase was especially pronounced in the South (759 reports), Northeast (711 reports), and Midwest (697 reports). Acetyl fentanyl, AH-7921, and MT-45 were first reported to NFLIS in 2013, whereas mitragynine was first reported in 2010. Acetyl fentanyl increased from 6 reports during the second half of 2013 to 55 reports during the first half of 2014. According to medical examiner data compiled by the CDC, 202,157 deaths were the result of a drug poisoning or overdose between 2009 and 2013. Of these deaths, 57% involved heroin and natural, semisynthetic, and synthetic opiates.

NFLIS publically shares data that can benefit management decisions of crime laboratories through various reports throughout the year, including special reports on such drug classes as presented in this presentation. NFLIS provides a resource for the community to identify and respond to drug trends.

Opiates, Regional Trends, National Estimates

B30 Statistical Analysis of Firearms: A Comparison Between the 2D and 3D Integrated Ballistic Identification System (IBIS®)

Keith B. Morris, PhD, 208 Oglebay Hall, 1600 University Avenue, PO Box 6121, Morgantown, WV 26506-6121; Roger Jefferys, BS*, 27 Dafonzo Hill Road, Pursglove, WV 26546; and Eric F. Law, BS*, 35 Metro Towers Lane, Apt 205, Morgantown, WV 26505*

After attending this presentation, attendees will understand how Bayesian networks can be used to estimate likelihood ratios based upon information available during a specific firearms-related investigation, how to interpret that data in order to obtain the best results for use in a court of law, and how to provide validity for the state and the accused.

This presentation will impact the forensic science community by providing a greater understanding of the means to interpret firearms evidence by using Bayesian networks which were developed from data retrieved from 2D and 3D IBIS®.

IBIS®, developed by Forensic Technology International (FTI), serves as the backbone of the National Integrated Ballistic Information Network (NIBIN) system.[1] This system allows for the databasing of images of cartridge cases and bullets.

The goal of this study was to perform a 3D IBIS® analysis and compare the results to that of a 2D IBIS® analysis. The intra- and inter-variation of the results were also analyzed. A West Virginia University (WVU) Legacy IBIS® and the new 3D FTI IBIS® were used during this study. The cartridge cases from a sample set of 12 9mm firearms were used to study 3D correlations with the cooperation of FTI. These 12 firearms were selected based on preliminary data which displayed their performances of Breechface (BF) and Firing Pin (FP) IBIS® scores via their Receiver Operating Characteristic (ROC) curves and the accompanying Area Under the Curve (AUC) values. ROC curves can be used to determine the crossovers between match and non-match. The ROC curve demonstrates the discriminating power of the method. In other words, it determines how well the method can differentiate between different states of the samples to which the method has been applied. This discriminating ability is directly related to the area under the ROC curve.

A Bayesian network was created to help compare IBIS® scores from the 2D and 3D IBIS® correlations using Netica®, a program for working with belief networks and influence diagrams.[2] Scatter plots, density distributions, and ROC curves were generated for the 2D and 3D data using RStudio®, a user interface for R[3], a computer programming language and environment for statistical computing and graphics.[4]

The worst discriminating power category from FTI with respect to all the firearms analyzed is 2D BF whereas the best discriminating power category is 3D FP. The worst discriminating power category from WVU with respect to all firearms analyzed is coincidentally the BF scores while the best is the FP. Comparing all of the data from both instruments, they behaved similarly, resulting in the worst performance resonating from a Ruger® LC9 in the category of 2D BF scores. Also noteworthy is the benefit of the addition of the side light feature for analyzing the BF. Overall, with regard to an added dimension (i.e., 2D vs. 3D), there was no significance in the results to conclude that one is better than the other.

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Firearms, IBIS®, Bayesian

B31 "I Dropped Acid." "No, You Didn't." A Retrospective Study of NBOMe Emergence in Harris County, Texas

Warren C. Samms, PhD, 1885 Old Spanish Trail, Houston, TX 77054; Donna E. Williams, BS, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; and Kay McClain, BS, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will better understand the growing availability and use of the hallucinogen class NBOMe within the largest county of Texas. These drugs continue to grow in popularity in southeast Texas, despite fatal overdoses reported in Texas and elsewhere in the United States.

This presentation will impact the forensic science community by showcasing a four-year emerging trend of a powerful and deadly class of hallucinogens. The data will show that users appeared not to know the identity or the danger of the substance they possessed. This information will be of use to other communities in helping to address emerging drug trends.

In the early 2010s, many "designer" substances appeared, including synthetic cannabinoids, substituted cathinones, and NBOMe, also known as "New LSD" hallucinogens, such as "25-I," "25-C," and "25-B." The drugs became available inexpensively online and in retail outlets, reaching a young demographic. This, coupled with the high-potency NBOMe compounds, caused a serious public health concern. Some of the first reported deaths from these potent hallucinogens were found in Harris County, Texas.

The Harris County Institute of Forensic Sciences Drug Chemistry Laboratory first encountered NBOMe hallucinogens in January 2012 as part of a large seizure of various stimulant, hallucinogen, and cannabinoid powders. Since then, the number of incoming NBOMe hallucinogens has increased each year: 19 items in 2012, 67 in 2013, and 115 in 2014. It has been identified on blotter paper, in liquid and, on a few occasions, in solid dosage forms. Most commonly, small quantities for personal use are submitted to the laboratory.

Given the increasing number of small-quantity possession cases, despite increasing media attention on the harmful and potentially fatal effects of these drugs, it is hypothesized that many of those people found in possession of NBOMe hallucinogens did not know the identity of the substance and did not understand the risk. It was speculated that suspects often believed that they possessed LSD.

The offense reports of all cases that had tested positive for one of the NBOMe hallucinogens over the period from 2012 to present were examined. Demographic data, the location of the seizure, and suspects' statements regarding the identity of the drug were gathered. These data were correlated with the charging information and the judicial outcome, and charging trends were looked for. As expected, suspects commonly claimed NBOMe was LSD or some other unrelated substance. Very few suspects mentioned "N-bomb," "New LSD," or "NBOMe." These and other details of the study will be discussed.

NBOMe, Designer Drugs, Retrospective

B32 A Study of Microcrystal Tests for Emerging Psychoactive Substances

Sean Brady, West Chester University of PA, West Chester, PA 19383; and Monica Joshi, PhD, West Chester University, Dept of Chemistry, Schmucker Science South, 750 S Church Street, West Chester, PA 19383*

After attending this presentation, attendees will be able to evaluate the role of microcrystal tests in the detection and identification of emerging psychoactive substances.

This presentation will impact the forensic science community by providing a renewed look at microcrystal tests as quick, simple, and effective steps in a drug identification process. This presentation describes the application and chemistry of traditional microcrystal test reagents for the identification of the new classes of psychoactive substances.

Microcrystal tests have held a place in drug identification schemes because they have features of both presumptive tests and confirmatory tests.[1] They are rapid, simple, and specific and can be used to discriminate between closely related analogs and isomers. The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) classifies microcrystal tests as a Category B technique, recognizing they have a greater discriminatory potential than other presumptive tests. Crystals with characteristic shape, habit, and optical activity are formed when the drug is mixed with a precipitating reagent to form specific drug-reagent complexes.[2] Microcrystal tests are not without their limitations. Factors such as concentration, drying time, and adulterants can cause distortion in the crystal shape and habit. The best tests are reliable, quick, and produce recognizable crystals reproducibly with a given drug, like the ones for cocaine and amphetamine. The American Society for Testing and Materials (ASTM) guidelines for cocaine and amphetamine identification have been used by many laboratories to identify these drugs in drug exhibits.

The rise in highly sophisticated instrumental techniques has caused a decline in the use and growth in the area of microcrystal testing; however, because of the advantages described above, it is worth evaluating their performance characteristics with emerging drugs. A quick survey of SWGDRUG monographs reveals that several of the new classes of drugs do not have microcrystal tests described for them. Recently, Elie at al. described the crystals for selected drugs using mercuric chloride as a reagent.[3] The study describes the benzylpiperazine test, mephedrone test, and crystals of potential interferences such as caffeine. The study demonstrates that microcrystal tests can be used to analyze new psychoactive substances with traditional reagents.

This presentation evaluates well-established reagents and their reactions with compounds representing five classes of emerging psychoactive substances: piperazines, phenethylamines, tryptamines, aminoindanes, and cathinones. Each psychoactive substance is thoroughly studied with reagents such as gold and platinum bromides, gold and platinum chlorides, mercuric chloride, and iodide. The presentation discusses the behavior of each analyte with specific reagents under different conditions as well as the selectivity, repeatability, and reproducibility of each test.

Overall, the reagents with gold and platinum showed consistent drug-reagent crystals. Some structurally similar compounds that gave the same crystal shape and habit can be differentiated by the dichroism observed under cross-polars. Different crystal characteristics were observed for the salt and freebase forms of compounds.

This study will help analysts determine if the microcrystal tests can be adopted in their current analytical scheme to complement the instrumental techniques.

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Microcrystal Tests, Drugs, Microscopy

B33 Sample Introduction Studies for Direct Analysis in Real-Time (DART®) Systems

Rachel Masek, BS, Eastern Kentucky University, 4112 NSB, 521 Lancaser Avenue, Richmond, KY 40475; Amelia Hartman, Eastern Kentucky University, 4112 NSB, 521 Lancaser Avenue, Richmond, KY 40475; and David Cunningham, PhD, 5142 NSB, 521 Lancaster Avenue, Richmond, KY 40475*

After attending this presentation, attendees will better understand the sample introduction methods currently available for DART® systems and designs with improved performance characteristics. Fundamental aspects of the DART® source ionization and optimization will be reviewed along with steps taken during the optimization of key instrumental parameters. Several examples of applications will be provided, including the analysis of trace drug residues on clothing.

This presentation will impact the forensic science community by providing a sound fundamental description of currently available methods for sample introduction into DART® systems. While DIP-It™ glass tips and QuickStrip™ cards provide rapid analysis options for some types of samples, additional options based on membranes and adhesive tapes will be presented. The potential advantages that these options offer for several types of samples, including drug residue on clothing and skin, will be described.

Sample introduction methods for DART® systems include DIP-It™ glass tips which are dipped into liquid solutions and QuickStrip™ cards which consist of a metal wire mesh that can be placed into powder with some sample adhering to the mesh.[1] Custom sample holders have been made from cotton tip swabs with introduction on a motorized rail system.[2] Results from these devices indicate that analyte signal is generally obtained from the edges of the device where ionized gasses can interact with the sample and the resulting analyte ions are allowed to flow unimpeded into the mass spectrometer. In fact, a cotton swab placed in front of the inlet may result in negligible signal due to blockage of the analyte ions.[2] The present work involves the design of materials with large edge surface areas and geometries conducive to high gas flow rates. Initial material screening studies will be presented that include monitoring of background ions generated by the DART® source ions under various conditions (discharge needle voltage, gas steam temperatures, etc.). Studies were performed using a Simplified Voltage and Pressure (SVP) ion source interfaced to a Linear Trap Quadropole (LTQ) XL™ linear ion trap mass spectrometer with data analysis using the Thermo Xcalibur™ software. Custom holders were constructed and placed inline between the DART® source outlet and the ceramic tube leading to the Vapur™ flange before the inlet to the mass spectrometer. Materials were identified that could readily transfer drug residues from clothing and skin for analysis. The new designs and materials allow facile transfer of trace residues for rapid analysis. The methods represent a substantial improvement to the uncontrolled "wanding" of a sample held by tweezers in front of the mass spectrometer inlet.

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DART®, Drug Analysis, Sample Preparation

B34 The Detection of Bleach (Sodium Hypochlorite) in Dialysis Blood Lines and Syringes in a Serial Murder Investigation

S. Frank Platek, MS, US FDA, Forensic Chemistry Center, 6751 Steger Drive, Cincinnati, OH 45237-3097; John B. Crowe, BS, US FDA Forensic Chemistry Center, 6751 Steger Drive, Cincinnati, OH 45237; and David S. Jackson, BS, US FDA Forensic Chemistry Center, 6751 Steger Drive, Cincinnati, OH 45237*

After attending this presentation, attendees will understand what analytical techniques can be used to detect bleach (sodium hypochlorite) in difficult matrices and how this information was used in court to convict a serial murderer for assaulting and murdering several patients at a dialysis facility.

This presentation will impact the forensic science community by shedding light on bleach detection in difficult matrices and the possibility for identifying bleach even when all of its active ingredients have broken down.

After a series of unexplained deaths and adverse reactions of patients at an eastern Texas dialysis center over a one month period, the state initiated an investigation that led to the arrest of a licensed practical nurse who worked at the center. The nurse was accused of murdering five dialysis patients and injuring five additional patients by injecting their dialysis blood lines with bleach (sodium hypochlorite) during their treatments.

The detection of bleach is challenging because sodium hypochlorite rapidly degrades in matrices that can be oxidized. Methodology to characterize bleach and its breakdown products in product tampering investigations has been developed at the Forensic Chemistry Center (FCC). This has been used to determine bleach adulteration in product tamperings even when all of the active hypochlorite has degraded. This is accomplished using a combination of spot tests for oxidizing agents, iodometric titration to assay sodium hypochlorite content, and ion chromatographic analysis for chloride and chlorate (bleach degradation products). In addition, headspace Gas Chromatography/Mass Spectrometry (GC/MS) has been used in some cases to further characterize the interaction of bleach with the sample matrix.

Eight dialysis blood lines and numerous needles and syringes from suspected victims at the dialysis clinic were received at the FCC for analysis. Liquid collected from various sites on the blood lines, residue on discarded syringe needles, and contents of suspect syringes were all analyzed for bleach and bleach degradation products. In addition, Fourier Transform Infrared (FTIR) analysis was used to test crystalline residues from suspect syringes to detect sodium hypochlorite and chlorate. Puncture analyses were performed on selected dialysis line injection ports to determine the number of punctures and to characterize the puncture holes as an indication of tampering. The analysis results were presented in court; the accused was found guilty on five counts of capital murder and three counts of aggravated assault.

Bleach (Sodium Hypochlorite), Serial Murder, Dialysis

B35 Comparison of the Restek Rtx®-5, Rxi®-1ms, and Rxi®-1HT Gas Chromatography (GC) Columns for the Qualitative Analysis of Synthetic Cannabinoids

Laurel A. Hardy, BS, 1412 7th Avenue, Apt #21, Huntington, WV 25701; Carrie J. Kirkpatrick, BS, 725 Jefferson Road, South Charleston, WV 25309; Pamela J. Staton, PhD, Marshall University Forensic Science MSFS & Center, 1401 Forensic Science Drive, Huntington, WV 25701; and Lauren L. Richards-Waugh, PhD, Marshall University, 1401 Forensic Science Drive, Huntington, WV 25701*

After attending this presentation, attendees will better understand the difficulties of synthetic cannabinoid analysis in a high-throughput setting and gain insight as to what GC columns could be put to use in the laboratory to improve the efficiency of synthetic cannabinoid analysis.

This presentation will impact the forensic science community by providing results from a comparison of synthetic cannabinoid qualitative analysis using the Restek Rtx®-5, Rxi®-1ms, and Rxi®-1HT GC columns. This research could impact law enforcement efforts by serving as a resource for all forensic drug laboratories searching for ways to make their analysis of synthetic cannabinoids more efficient.

As popularity of synthetic cannabinoids and the prevalence of their harmful side effects grow, so does the need to control such substances. For high-throughput laboratories such as the West Virginia State Police (WVSP) Drug Identification Laboratory, the high molecular weight and low volatility of synthetic cannabinoids poses a problem for analysis as not all synthetic cannabinoids elute within the parameters of their standard Gas Chromatography/Mass Spectrometry (GC/MS) method. This study compares the Restek Rxi®-1ms and Rxi®-1HT GC columns to the Restek Rtx®-5 GC column (standard in the WVSP Drug Laboratory) to determine if either column could improve the efficiency of synthetic cannabinoid analysis using the standard GC/MS method.

A total of 53 synthetic cannabinoid standards were analyzed and the results indicated a dramatic decrease in retention time (average of 2.106 minutes) when using the Restek Rxi®-1HT GC column for analysis and a slight decrease in retention time (average of 0.488 minutes) when using the Restek Rxi®-1ms GC column for analysis. Data from both the Restek Rxi®-1ms and Rxi®-1HT columns were determined to be statistically significantly different from data obtained using the Restek Rtx®-5 column, based on paired *t*-tests with 95% confidence intervals. Both columns demonstrated adequate reproducibility of retention time for the qualitative analysis purposes of the WVSP Drug Identification Laboratory. In conclusion, the Restek Rxi®-1HT and Rxi®-1ms columns proved to be a promising possibility for the qualitative analysis of synthetic cannabinoids in high-throughput laboratories.

Synthetic Cannabinoid, Gas Chromatography, Qualitative

B36 Colorimetric-Based Paper Microfluidic Devices for the Presumptive Determination of Seized Drugs

Ling Wang, MS, Florida International University, CP-304 11200 SW 8th Street, Miami, FL 33199; Bruce R. McCord, PhD, Florida International University, Dept of Chemistry, University Park, Miami, FL 33199; Giacomo Musile, PhD, Università Di Verona, p.le Scuro 10, Dept Legal Medicine, Verona 37134, ITALY; Jashaun Bottoms, Tuskegee University, 1200 W Montgomery Road, Tuskegee, AL 36088; and Franco Tagliaro, PhD, MD, Dept of Medicine & Public Health, Policlinico GB Rossi, P. le Scuro No. 10, Verona 37134, ITALY*

The goal of this presentation is to describe the development of colorimetric-based paper microfluidic devices for the presumptive determination of seized drugs. Information provided will include the optimized design of a multi-channel paper microfluidic device, the multiplexed detection of different controlled substances, and the development validation of the multi-channel paper chip.

This presentation will impact the forensic science community by demonstrating the application of this newly designed paper microfluidic device in the presumptive detection of seized drugs. The new method is rapid, inexpensive, and applicable to a wide variety of seized drugs.

Colorimetric reagents have been used for testing seized drugs for many years. Although these reagents provide a useful presumptive determination, they are less convenient and more dangerous because of the presence of toxic and corrosive chemicals. This research provides an alternative platform for colorimetric detection based on paper microfluidics. To this end, six-channel chips were created that adapt these colorimetric reagents to a multiplexed ready-to-use format. Each lane performs a different test. In the field, samples are dissolved in a carrier solvent in vials, then applied to the paper just prior to analysis. These devices can be used at crime scenes, laboratories, and any other locations where seized drugs need detection. These paper microfluidic devices are easy to prepare, inexpensive to operate, and can be conveniently stored for later use with shelf lives of two to three months.

The paper microfluidic devices are designed as a six-channel multiplexed system. Preparation of the devices requires a wax-ink printer, thermal laminator, chromatography paper, and colorimetric reagents. The wax-ink printer and a thermal laminator produce hydrophilic channels defined by melted wax on the paper. Next, a variety of different colorimetric reagents are prepared and a different test is prepared for each channel to create six simultaneous and separate detection zones. Drugs in powder form are dissolved in solutions then transferred to the chips where they move to the detection zone via capillary action. Sequences of different reagents can be applied to each channel to produce a series of reactions and the color changes appear at the end of each channel. The entire process takes less than five minutes. Because each specific drug can produce a color change that depends on the specific reagent in each channel, it becomes possible to presumptively determine the type of drug in the test solution.

One important aspect of this study is the selection of potential reagents for the device. Traditional colorimetric reagents, such as the Mandelin and Froehde reagents, use concentrated sulfuric acids. Acids such as sulfuric acid and nitric acid can burn and digest chromatographic paper. As a result, a variety of chemical tests were performed to modify these reagents in order to make them more compatible with the paper-based format. For example, potassium manganate (VII), copper (II) sulfate, and iron (III) have been utilized in various forms to create alternate colorimetric reagents. The adjusted reagents produce specific color changes for seized drugs on the paper microfluidic devices. Procedures have been developed for the detection of cocaine, ketamine, codeine, ephedrine, morphine, amphetamine, methamphetamine, and MDMA. These devices have been tested for sensitivity, specificity, and stability against a variety of potential interferences and test conditions.

The use of paper microfluidic devices permits the development of rapid, inexpensive, and easily operated tests for a variety of seized drugs. They present a safe and convenient presumptive tool for samples that can be used in the field, prior to confirmatory laboratory analysis.

Paper Microfluidic Devices, Colorimetric Reagents, Seized Drugs

B37 Evaluation of Microscopy and Vibrational Spectroscopy for the Discrimination of Purple and Blue Nail Polishes

Brianna Kroon, 4333 Wells Curtice Road, Canandaigua, NY 14424; Elaine M. Pagliaro, JD, University of New Haven, Lee Institute of Forensic Science, 300 Boston Post Road, West Haven, CT 06516; and Brooke W. Kammrath, PhD, University of New Haven, Forensic Science Dept, 300 Boston Post Road, West Haven, CT 06516*

After attending this presentation, attendees will understand the discriminating power of microscopic and spectroscopic analytical methods for the analysis of blue and purple nail polishes.

This presentation will impact the forensic science community by evaluating the discriminating potential of microscopic and spectroscopic methods for the analysis of blue and purple nail polishes.

Nail polish is a common and popular quick-drying lacquer that is painted on fingernails or toenails for aesthetic purposes. Nail polish falls under the category of cosmetic evidence and limited research has been performed regarding the evaluation of microscopy (stereomicroscopy, brightfield, and polarized light) and spectroscopy (infrared and Raman) as these techniques relate to nail polish as cosmetic evidence. Although identification and discrimination of nail polish is not commonly practiced by forensic scientists, cosmetic evidence and, more specifically, nail polish has played a key role in criminal cases. Most infamously, nail polish was valuable forensic evidence in the "Wood Chipper Murder Case" in Connecticut. Nail polish is either a transparent or colored lacquer that contains the following basic components: film forming agent, resins or plasticizers, solvents, and coloring agents. Each component has a different purpose: the film-forming agent creates a protective layer over the polish; the plasticizer or resin improves flexibility of the nail polish and makes the nail polish more resistant to water and soap; the volatile solvents hold the mixture of materials and colorings until the polish is applied; and the coloring agent, which is comprised of organic dyes or inorganic pigments, contributes to the overall color of the nail polish.

Nail care is a large part of the cosmetic industry, with global sales in 2014 estimated to be nearly $1.2 billion. Nail art not only follows trends but is also viewed as a form of personal expression, which is why there is a plethora of available colors. This study focused on blue and purple nail polishes, which have not yet been studied by the forensic science community.

The discrimination power of microscopic and spectroscopic methods was evaluated in this research. Seven different brands of nail polish with seven different shades of purple and blue per brand of nail polish were analyzed. The shade of blue and purple were chosen to be as similar as possible between the brands, and a variety of brands were chosen to represent a selection of salon-quality polishes as well as polishes intended for at-home use. A total of 49 different polishes were analyzed using three types of microscopy (stereomicroscopy, brightfield, and polarized light microscopy) and two types of spectroscopy (Raman and attenuated total reflection Fourier Transform infrared microspectroscopy) in order to determine whether these methods could provide discrimination between the 49 bottles of blue and purple nail polish and/or brand identification.

All 49 bottles of nail polish were able to be discriminated microscopically, based on various pigment characteristics (i.e., size, dispersion, density, color, etc.), as were the presence of distinct effect pigments in some of the samples. Raman spectroscopy was successful in identifying some pigments in the polishes, specifically Pigment White 6 (anatase) and Pigment Blue 27; however, there was fluorescence in several samples that prevented pigmentation identification for every blue and purple polish. Infrared spectroscopy was used for brand identification, with Principal Component Analysis/Canonical Variate Analysis (PCA/CVA) hold-one-out cross validation proving to have a 1.9% error rate. The results from this research provide valuable information about cosmetic evidence that criminalists can use in investigations and adjudications.

Nail Polish, Microscopy, Spectroscopy

B38 Characterization by Scanning Electron Microscopy With Energy-Dispersive X-Ray Spectroscopy (SEM/EDX) of Nail and Gel Polishes and Its Real-World Applications

Audriana M. Wagner, University of New Haven, 300 Boston Post Road, West Haven, CT 06516; R. Christopher O'Brien, PhD, University of New Haven, Dept of Forensic Science, 300 Boston Post Road, West Haven, CT 06516; Elaine M. Pagliaro, JD, University of New Haven, Lee Institute of Forensic Science, 300 Boston Post Road, West Haven, CT 06516; and Brooke W. Kammrath, PhD, University of New Haven, Forensic Science Dept, 300 Boston Post Road, West Haven, CT 06516*

After attending this presentation, attendees will better understand the characterization and discrimination power of SEM/EDX for the forensic analysis of nail and gel polishes. In addition, real-world samples degraded in air, static freshwater, and dynamic freshwater environments were analyzed to assess changes in their elemental composition.

This presentation will impact the forensic science community by determining the characterizing and discriminating potential of SEM/EDX for the forensic analysis of nail and gel polishes as well as evaluating the effects of degradation on those results.

Because of their wide availability, popularity, and durability, nail polishes are an important type of cosmetic evidence. Although cosmetic evidence is not widely analyzed by forensic scientists, these types of evidence have proved to be incredibly valuable in a plethora of cases including the infamous "Wood Chipper Murder Case" in Connecticut. Traces of nail polish can be left at a location or transferred between individuals in a variety of ways, and the ability to associate a sample with the source (bottle and/or brand) is of the utmost importance.

SEM/EDX instrumentation is not only valuable for viewing powerful high-resolution microscopic images of samples but also for measuring their elemental composition. This instrumentation is commonly used to analyze trace paint evidence gathered from the scenes of hit-and-runs or burglaries for identification purposes. Nail and gel polish composition is complex and actually very similar to that of paint, which is the impetus for evaluating SEM/EDX performance in terms of nail and gel polish characterization and identification.

In this study, the effectiveness of SEM/EDX instrumentation was explored for the characterization of visually similar nail and gel polishes in red and pink hues. Seven different brands were chosen for each type of polish for a total of 14 brands. From each brand, seven colors of similar shades were selected as samples resulting in a total of 124 analyzed polishes including top and base coats from each brand. The brands used were a mix of salon quality polishes and polishes intended for at-home use. Additionally, painted nail clippings underwent extensive degradation studies in air, static freshwater, and dynamic freshwater environments, which were similarly analyzed by SEM/EDX to measure the degradation effects from each environment over a time period of one month. These degradation studies of painted human fingernails mimicked submissions of evidence to a forensic laboratory and offer real-world information on the decay of nail and gel polishes in the tested environments.

This study evaluated the forensic relevance of SEM/EDX instrumentation for the analysis of nail and gel polishes by comparing elemental compositions of the selected polishes. Multivariate statistical analysis methods, such as Principal Component Analysis/Canonical Variate Analysis (PCA/CVA), pair-wise comparisons, and standard deviation match criteria were used to assess the discrimination ability of the instrumental methods used for both characterization and degraded samples. Results indicate that although brands and bottles could not be uniquely identified because of similar elemental profiles, this technique would be useful for exclusion. This research provides valuable information to the field of forensic science, specifically for the analysis of cosmetic evidence, where a gap in the literature exists.

Cosmetic Evidence, Forensic Science, SEM/EDX

B39 The Analysis and Classification of Tire Rubber Deposits Using Pyrolysis-Gas Chromatography/ Mass Spectrometry (Py-GC/MS)

Rebecca Thielen, BS, 427 N Milton Avenue, Campbell, CA 95008*

After attending this presentation, attendees will better understand a successful recovery method for tire rubber deposits left as a result of skidding or braking incidents at the scene of a crime, the role that road surface plays as a contaminant, and the ability to associate these rubber deposits with the tire from which they originated.

This presentation will impact the forensic science community by contributing valuable and novel information on a topic with little previous research, none of which was previously conducted in the United States. This presentation will encourage forensic scientists to recognize this as a useful type of evidence within the forensic trace field as well as demonstrate an effective method for tire analysis that can be performed in crime laboratories.

Many crimes involve the use of motor vehicles and, as a result, impressions or tire deposits may be left behind. This type of evidence has the ability to provide forensic scientists with information about a vehicle's tires, such as tread pattern and brand, and therefore possibly information about the vehicle size or type.[1] Oftentimes, these impressions and markings are not of the best quality and provide very little probative information. In these cases, it would be beneficial to be able to analyze tire rubber deposits left at a scene; however, comprehensive studies have not been done on this topic. Previous research has identified Py-GC/MS as a successful method in the analysis of rubber deposits. These studies have shown this instrument's ability to distinguish between different manufacturers of tires as well as between different tire models of the same manufacturer on the basis of tire tread chemical composition.[2,3] Other studies have confirmed these findings, as well as applied statistics to help determine that there is low intra-variability within each tire and a high enough inter-variability between tires to correctly assign deposits to their source tire.[4] While this research has explored some sample collection methods and has acknowledged the possibility of road surface contamination, neither have been comprehensively studied.

In this experiment, tire deposits were made on both concrete and asphalt by eight different vehicles, each with a different brand or model of tire. Immediately following each deposit, both concrete and asphalt surfaces were tape lifted separately to collect any rubber deposits left behind. A thin slice of tread was removed from four different areas of each tire that made a deposit. Using a stereomicroscope, each tape lift was examined and rubber deposits were removed with tweezers. Each was analyzed separately using a validated rubber method adapted from previous research on the Py-GC/MS.[4,5] Four tire tread samples from the tire that made the corresponding tire deposit were then run on the Py-GC/MS. Control samples of the asphalt and concrete were collected and analyzed to account for any contamination. The resulting chromatograms were superimposed and compared to study retention times and overall peak patterns. Then, Target Compound Identification (TCI), normalization, and peak area were used to determine whether different tires had distinguishing chromatograms and if tire deposits had the same chromatograms as their corresponding tire tread samples.

Extracted ions and TCI were used to classify each sample based on its primary rubber content. Six of the eight samples were classified as Styrene-Butadiene Rubber (SBR) and the other two as a mix of SBR and Natural Rubber (NR). The data was normalized to the styrene peak for SBR samples and the limonene peak for SBR/NR samples. In addition to normalization, the peak area, relative intensity, and extracted ions were used to help further classify each group based on the presence of small amounts of additives that differ between tire models and brands. Concrete and asphalt surfaces do contribute a small amount of contamination, but it does not interfere in the association of the deposit to the tire from which it originated. This preliminary analysis of the data reveals two important conclusions: (1) there are noticeable differences in chemical composition between different brands and models of tires; and, (2) tire deposits have the same composition as the tire tread samples from which they originated. The use of tape lifts as a recovery method in this study proved to be successful compared to other methods tested. The tape lifts collected a sufficient amount of tire residue for analysis without any significant interference from the adhesive. This method worked even in situations in which cars left very light deposits behind.

In conclusion, this study demonstrates that not only can different types and models of tires be differentiated from one another, but their rubber deposits can also be differentiated from one another as well as associated to the tire from which they originated. The findings from this research could be used to develop specific tire deposit recovery methods to be used during evidence collection as well as integrate tire rubber analysis as an examination that crime laboratories perform.

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Rubber Deposits, Tires, Pyrolysis

B40 Organization of Scientific Area Committees (OSAC) Activities Impacting Laboratory Operations

John P. Jones II, MBA, National Institute of Standards & Technology, 100 Bureau Drive, MS 8102, Gaithersburg, MD 20899*

After attending this presentation, attendees will understand the latest standards and guidelines reviewed by the OSAC for Forensic Science and their potential to impact laboratory operations. The OSAC is comprised of 33 operating units and more than 100 task groups populated by 540 OSAC members and 150 affiliates, all working on specific standards activities.

This presentation will impact the forensic science community by educating attendees on the standards and guidelines reviewed by the OSAC and how implementation of these standards in the forensic science community is likely to occur. Instructions will also be provided on how individuals can become involved with the OSAC and can have an impact on standards development efforts.

The OSAC has been fully operational since January 2015 and continues to transition the fragmented standards development efforts in the forensic industry to a unified process of recognizing valuable standards and guidelines that have both scientific merit and wide-based community acceptance. OSAC subcommittees have reviewed an initial National Institute of Standards and Technology (NIST) -developed catalog of external standards and guidelines that contained more than 700 documents that existed prior to OSAC. As part of the OSAC development and review process, subcommittees determine if documents have scientific merit and if they were developed through an open standards development process. In addition, documents are reviewed to determine if they have addressed legal and human factors issues and have properly documented the potential impact on existing laboratory operations. From this effort, the 24 OSAC subcommittees have selected specific documents to route for approval to post on OSAC registries or, in many cases, to further develop with the intention of enhancing a document's scientific basis and/or range of public input received.

With forensic science practitioners, researchers, statisticians, and measurement scientists collaborating to bolster existing standards and guidelines or develop new standards from scratch, the forensic science community can have confidence in the documents that are approved to be listed on the *OSAC Registry of Approved Standards* and the *OSAC Registry of Approved Guidelines*.

Standards, OSAC, NIST

B41 Organization of Scientific Area Committees (OSAC) — Increasing Visibility of Standards in Forensic Science and the Potential Impact in the Laboratory and the Courtroom

Mark D. Stolorow, MS, MBA, NIST Special Programs Office, Organization of Scientific Area Committees, 100 Bureau Drive, MS 8102, Gaithersburg, MD 20899-8102*

After attending this presentation, attendees will understand how the efforts of more than 700 subject matter experts (volunteers) appointed to the OSAC for forensic science and others could impact laboratory protocols, accreditation efforts, reports, expert testimony, and criminal justice proceedings.

This presentation will impact the forensic science community by illustrating how, as judges, prosecutors, and defense attorneys become acquainted with the *OSAC Registry of Approved Standards* and *OSAC Registry of Approved Guidelines*, direct and cross examination of expert witnesses will increasingly examine conformance with the published standards and guidelines used in conducting the forensic analysis and interpreting data. Expert witnesses will increasingly need to confirm in testimony the scientific validity of their protocols and candidly share with juries any limitations of their analyses and interpretations.

The OSAC design employs the essential requirements of developing consensus-based standards, which include openness, transparency, balance of interest, due process, and an appeals process that ensures each stakeholder's viewpoints are properly considered. In addition, the OSAC infrastructure brings a uniform standards recognition platform to the community, enhances scientific rigor, and increases communication among forensic scientists, research scientists, academicians, statisticians, attorneys, managers, and quality-assurance specialists. The OSAC structure consists of a Forensic Science Standards Board, three resource committees, five scientific area committees, and 24 subcommittees.

This presentation will impact the forensic science community by educating attendees on the processes employed by the OSAC to identify, foster development, and formally approve forensic science standards through publication on the *OSAC Registry of Approved Standards* and *OSAC Registry of Approved Guidelines*. These standards and guidelines will be implemented voluntarily by practitioners and incorporated into auditing processes by accreditation bodies. The high visibility of approved standards and guidelines on the OSAC registries will ultimately impact quality standards for report writing and expert testimony in the courtroom.

As the forensic science community is aware, the development of a quality infrastructure for forensic science was a key component of some of the reforms anticipated in the 2009 National Academy of Sciences (NAS) Report, Strengthening Forensic Science in the United States – A Path Forward. OSAC is now operational and beginning to populate the OSAC registries with approved standards and guidelines. As judges, prosecutors, and defense attorneys become acquainted with the *OSAC Registry of Approved Standards* and *OSAC Registry of Approved Guidelines*, direct and cross examination of expert witnesses will increasingly examine conformance with the published standards and guidelines used in conducting the forensic analysis and interpreting data. Expert witnesses will increasingly need to confirm in testimony the scientific validity of their protocols and candidly share with juries any limitations of their analyses and interpretations.

The consensus-based documentary standards and guidelines approved for posting on the OSAC registries will be considered by laboratory directors and quality-assurance managers as standard methods for specific analyses. Accreditation bodies will consider the published discipline-specific standards for incorporation into their International Organization for Standardization (ISO) 17025 supplemental standards. As forensic science practitioners increase employment of quantification, uncertainty measurements, and probabilistic models in casework, there will also be increased utilization of quantitative results and probabilistic data in laboratory reports and expert testimony.

Standards and Guidelines, OSAC, Accreditation

B42 Chemometric Analysis of Gasoline Samples Utilizing Direct Analysis in Real-Time Mass Spectrometry (DART®-MS)

Ashley Davis, MS, Boston University School of Medicine, 72 E Concord Street, Boston, MA 02118; Matthew Pavlovich, PhD, Northeastern University, 360 Huntington Avenue, 140 The Fenway, Boston, MA 02115; Joseph H. LaPointe, BSc, IonSense, Inc., 999 Broadway, Ste 404, Saugus, MA 01906; Brian Musselman, PhD, IonSense, Inc., 999 Broadway, Ste 404, Saugus, MA 10906; and Adam B. Hall, PhD, Northeastern University, 360 Huntington Avenue, 140 The Fenway, 421TF, Boston, MA 02115*

After attending this presentation, attendees will better understand current and past efforts to provide brand differentiation of gasoline, a common ignitable liquid utilized by arsonists worldwide. In this research, DART®-MS was employed as an analytical approach for the analysis of gasoline in an effort to generate chemical attribute signatures for a wide variety of gasoline brands at various states of weathering.

This presentation will impact the forensic science community by providing information pertaining to current research applicable to the fire debris analysis community. This data shows that even in highly evaporated samples (75% and >90%), unique ions can be used to differentiate common gasoline brands from one another in a fraction of the total analysis time in comparison to current analytical approaches.

Gasoline is an easily obtainable ignitable liquid that arsonists commonly use to initiate or expedite the spread of an intentionally set fire. Current methods for the extraction and concentration of ignitable liquids from fire debris utilize passive headspace concentration with activated carbon strips. Typical extractions are conducted between 60°C-80°C for 12-16 hours based on American Society for Testing and Materials (ASTM) guidelines followed by Gas Chromatography/Mass Spectrometry (GC/MS) analysis. Normally, hundreds of low molecular weight hydrocarbons are detected resulting in chromatograms showing distinctive patterns characteristic of various ignitable liquid classes. While traditional GC/MS methods are sensitive and generate data, which can be classified as gasoline based on the ASTM classification scheme, they do not allow for differentiation of brands of gasoline (especially weathered samples) or detect higher mass ions that may permit the determination of unique chemical attribute signatures. Gasoline samples were obtained from various vendors including Shell®, Sunoco®, Irving®, Gulf®, and Cumberland Farms®. An evaporation series was created for each of the brands and analyzed utilizing QuickStrip™ cards by DART®-MS and also by GC/MS methods. The goals and objectives of this research were to optimize the DART®-MS parameters for gasoline analysis, evaluate the potential for DART®-MS to distinguish gasolines by brand, develop chemometric models to appropriately classify gasoline samples, and finally lay the groundwork for future studies that could further develop a more efficient and discriminating DART®-MS gasoline analysis method for forensic casework.

Preliminary studies using DART®-MS to analyze weathered gasoline samples using desorption ionization produced characteristic spectra for various brands of gasoline. Significantly, unique ions were detected in the higher mass range of the spectrum (>m/z 500). Using an ion trap mass analyzer and a scan range of 50amu-1,000amu, mass spectra rich in various ions were detected. Many of these ions are likely gasoline additives and are non-hydrocarbon in their nature. The data shows that DART®-MS detected higher mass ions not observed in the GC/MS data and also showed differential spectra for the varying gasoline brands. Principle Component Analysis (PCA) plots of the data created through these methods have shown that gasoline brands tend to cluster separately from one another, despite the extent of weathering as represented by the evaporation percentage. Using these unique ions and advanced chemometric analysis, statistical analysis software was used to build models that can discriminate analyzed samples in a fraction of the time in comparison to a traditional GC/MS approach. Although variables including the season of purchase, storage time, dilution, and age of the gasoline were observed to contribute to the resulting mass spectral data, once the mass spectra are further evaluated, they could offer even more discriminating power between samples in addition to brand identification. Techniques such as DART®-MS in combination with chemometric approaches could enable forensic laboratories to confidently identify questioned gasoline samples by brand in the future.

Fire Debris, Chemometrics, (DART®-MS)

B43 Using Atmospheric Pressure Chemical Ionization/Mass Spectrometry (APCI/MS) and Flow Injection for the Screening of Arson Accelerants

Clare M. Fried, BS, Cedar Crest College, 100 College Drive, Allentown, PA 18104; Thomas H. Pritchett, MS, 100 College Drive, Allentown, PA 18104; and Michelle Shortell, MS, Pennsylvania State Police, Bethlehem Regional Laboratory, 2932 Airport Road, Bethlehem, PA 18017*

After attending this presentation, attendees will understand a new approach to fire debris analysis and how carbon disulfide, in combination with an APCI source, contributes to a more efficient screening process.[1]

This presentation will impact the forensic science community by providing a new perspective on accelerant detection methods. Some advantages to this method include: (1) the MS-only and precursor scans for the same compound classes as in the American Society for Testing and Materials (ASTM) methods only take minutes to perform; (2) carbon disulfide, the solvent of choice for extraction with carbon strips and passive headspace analysis, has shown the ability as an efficient charge transfer agent in APCI with hydrocarbons; and, (3) using Tandem Mass Spectrometry (MS/MS) analysis provides prominent molecular ion peaks in Q1 scans, as well as provides precursor scans, that identify the same compound classes as the standard Gas Chromatography/ Mass Spectrometry (GC/MS) -extracted ion chromatograms.[2,3]

Every year in the United States, millions of dollars and thousands of businesses and private properties are lost due to intentionally set fires. The forensic fire debris field is a continuously shifting one, which presents constant challenges to those who are involved with the investigations. Arson investigations depend largely on quick detection and determination of ignitable liquids and their residues. Arson fires affect many people across the United States each year and the people responsible for deliberately setting the fires should be caught and held responsible for their crimes.[4,5]

In this study, an APCI-MS/MS method has been developed which has been used to screen common ignitable liquids. A passive headspace sampling technique, along with activated charcoal strips, were used to collect samples. A carbon disulfide reagent was added to each strip once the headspace was collected. The carbon disulfide extract was injected into the MS/MS using a flow injection technique. The MS/MS, an ABI® SCIEX™ 3200 Qtrap® triple quadrupole mass spectrometer, utilized an APCI source and positive-ion mode. A peak-hopping scan mode was employed, along with a step size of 1amu. A Q1 scan and precursor scans were run for each accelerant sample.

Ignitable liquids such as gasoline, diesel, lighter fluid, mineral spirits, turpentine, paint thinner, WD-40®, and kerosene were sampled. Prominent molecular ion peaks provided indications that each accelerant presented a different profile. Five different commercial gasolines were studied as well as four different commercial diesels. The gasoline samples presented similar profiles to each other. The diesel samples presented similar profiles as well. Precursor scans at 91amu and 128amu provided an insight into what contributed to prominent peaks seen in each accelerant sample. Sample profiles were completed using mass range binning and peak intensity sums to create bar graphs for each.[6]

In conclusion, this method could potentially be used in forensic fire debris analysis to screen for accelerants. This method could shorten analysis time considerably. According to the ASTM Standard, GC/MS methods include a total run time of 25.0 minutes.[7] This method cuts down run time to less than three minutes, with no cool-down time.

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Fire Debris, Accelerants, APCI

B44 Practical Methods for Prohibiting Microbial Degradation of Ignitable Liquids in Soil Samples

James Hoult, BS, 2100 Paramont Way, Modesto, CA 95355; and Katherine D. Hutches, PhD, ATF, 355 N Wiget Lane, Walnut Creek, CA 94598*

After attending this presentation, attendees will: (1) understand how ignitable liquids found in soil are degraded; (2) understand why this type of evidence should be refrigerated to prevent degradation; and, (3) be aware of new possible, practical alternatives to refrigeration.

This presentation will impact the forensic science community by offering arson investigators and analysts a new practical alternative to refrigeration to prevent microbial degradation of ignitable liquids. Prohibiting the degradation of ignitable liquids before they can be analyzed in the laboratory will increase the odds that ignitable liquids in problematic samples will be identified using Gas Chromatography/Mass Spectrometry (GC/MS), even when refrigeration is not possible.

It has been well established that microbes in soil and moldy building materials can degrade ignitable liquids through preferential degradation of select compounds.[1,2] Refrigerating soil samples with potential ignitable liquid samples has been found to slow microbial degradation, but this requires large amounts of refrigerated space that are expensive and not always available.[3] Ignitable liquid samples found in soil can also become degraded prior to reaching a laboratory due to the fast rate of degradation. The use of triclosan in place of refrigeration is an exciting development, but pure triclosan is not readily available to fire investigators, would need mixing in the field, and could have possible negative health effects.[3]

Carbon dioxide canisters, dry ice, and oxygen-absorbing pouches were chosen as possible practical alternatives to triclosan. These experiments sought to make the atmosphere in the sampling cans more anaerobic by reducing the amount of oxygen present or replacing the air with carbon dioxide. This is ideal because anaerobic metabolism of ignitable liquids has been shown to be less efficient than aerobic metabolism.[4]

To test if carbon dioxide canisters could prohibit microbial degradation, an adjustable bicycle tire inflator was used to displace the air in the cans with carbon dioxide. Positive samples were spiked with 20µL of gasoline or diesel. The cans treated with carbon dioxide were compared to spiked soil samples that were kept at room temperature or in a freezer. In early experiments, the addition of carbon dioxide appeared to prohibit microbial degradation; however, with further testing it was shown that carbon dioxide canisters are unreliable as a means of prohibiting microbial degradation. It was also evident that there was wide variation in the canisters' contents.

Dry ice was also tested as a possible alternative to the carbon dioxide canisters. Dry ice offered less potential contamination than carbon dioxide canisters and cooling of the soil to help prohibit degradation; however, the sublimation of the solid dry ice to gas caused the pressure in the cans to increase and several lids exploded off the cans. The small amounts of dry ice that could safely be used failed to stop microbial degradation.

Finally, Oxy-Sorb® pouches were added to cans of soil spiked with gasoline. Oxy-Sorb® pouches are used to keep food fresh by reducing the amount of oxygen that is present.[5] The pouches are iron-based oxygen scavengers that reduce the available oxygen due to the reaction of oxygen with ferrous iron.[5] The samples with Oxy-Sorb® pouches added were compared to gasoline-spiked soil samples that were kept at room temperature. The Oxy-Sorb® pouches prohibited microbial degradation for 28 days.

Oxy-Sorb® pouches are a promising alternative to refrigeration and could be used in the future to prohibit microbial degradation. Before the Oxy-Sorb® pouches can be reliably used to stop microbial degradation, further experiments are needed using ignitable liquids other than gasoline. Other brands and sizes of oxygen-scavenging pouches could also be explored.

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Fire Debris, Microbial Degradation, Ignitable Liquid

B45 The Surprising Effect of Temperature on the Weathering of Gasoline

Heather Birks, BS, Virginia Commonwealth University, 1015 Floyd Avenue, Rm 2015, Richmond, VA 23284; Ashley Cochran, BS*, West Virginia University, Oglebay Hall, Morgantown, WV 26505; Tyler Williams, 98 Hearthstone Way, Hanover, MA 02339; and Glen P. Jackson, PhD, West Virginia University, Dept of Forensic and Investigative Science, 208 Oglebay Hall, Morgantown, WV 26506-6121*

After attending this presentation, attendees will understand how the evaporation temperature affects the distribution of remaining compounds after gasoline samples have been weathered to simulate fire debris samples.

This presentation will impact the forensic science community by providing practitioners with an explanation of why gasoline samples often do not appear to be evaporated to the same extent as one might expect based on the very high temperatures of structure fires.

Arson investigations often involve the identification and characterization of ignitable liquid residues in fire debris. The most commonly used ignitable liquid is gasoline because it is so readily available and particularly effective. Gasoline often evaporates from its containers during storage as well as through exposure to high temperatures, such as during a fire. Such evaporation is often termed weathering, and it is widely accepted that ignitable liquid residues are more likely to resemble weathered liquid samples than pristine liquid samples.

Prior research has investigated the weathering characteristics of gasoline under a variety of experimental conditions, but to date this has been limited to basic pattern recognition of the resulting chromatograms. Relatively little work has been performed to understand how weathering conditions — such as temperature, pressure, or convection — affect the distribution of the various classes of volatile components. For the current study, gasoline samples were weathered at atmospheric pressure using a stream of nitrogen gas and under vacuum. At both pressures, samples were weathered to 75%, 90%, and 95% by weight and at three different temperatures of 25°C, 60°C, and 90°C. After weathering, samples were analyzed using an Agilent® Gas Chromatograph/Mass Spectrometer (GC/MS) with conventional conditions on a 30m DB5 column. The resulting chromatograms showed that the earliest eluting, most volatile compounds — like toluene and the C_2-alkyl benzenes — tend to remain at significantly ($\alpha<0.05$) higher levels at higher temperatures relative to weathering to the same level at lower temperatures.

For example, at 75% weathering, samples weathered at 90°C had toluene and C_2-alkyl benzene peaks that were three to four times more abundant than samples weathered at room temperature ($\alpha\sim1x10^{-7}$, $n=20$). At 90% and 95% weathering, the same peaks were typically below threshold when weathered at 25°C and 60°C, but were typically above threshold when weathered at 90°C. The use of nitrogen had a small effect on weathering, but was insignificant relative to the temperature effect. The consequence is that samples weathered at higher temperatures appear less weathered (on a percent level) than one would expect. These results are consistent with the known relationships between vapor pressure and temperature for the different compounds. The relationship between vapor pressure and temperature was further validated by weathering of a simplified synthetic mixture of seven compounds found in gasoline, and the simulated (purely theoretical) weathering of the same compounds based on published Antoine constants.

This study may help explain why real fires often cause gasoline residues to appear far less weathered than one would expect based only on the expected weathering temperatures.

Arson, Gasoline, Weathering

B46 Mathematically Modeling Chromatograms of Evaporated Ignitable Liquids for Fire Debris Applications

Rebecca J. Brehe, BS, 6180 Bluff Road, Washington, MO 63090; John W. McIlroy, PhD, Drug Enforcement Administration, 10150 Technology Boulevard, E, Dallas, TX 75220; Ruth Waddell Smith, PhD, Michigan State University, School of Criminal Justice, 560 Baker Hall, East Lansing, MI 48824; and Victoria L. McGuffin, PhD, Michigan State University, Dept of Chemistry, East Lansing, MI 48824-1322*

After attending this presentation, attendees will be familiar with a mathematical model that can be used to generate chromatograms of ignitable liquids evaporated to different levels. The mathematically generated chromatograms can be used to populate reference databases that are used to aid in the identification of ignitable liquids in fire debris samples.

This presentation will impact the forensic science community by providing a method to generate the chromatogram of an ignitable liquid at any level of evaporation, thereby overcoming the variable and time-consuming nature of evaporating ignitable liquids experimentally.

Fire debris samples are typically extracted and analyzed by Gas Chromatography/Mass Spectrometry (GC/MS) to identify any ignitable liquid present. Identification is based on comparison of chromatograms to a database of ignitable liquid reference standards with mass spectral interpretation to identify specific compounds. To account for chemical changes in the liquid that occur during the fire, reference standards experimentally evaporated to different levels are often included in these databases; however, there are numerous variables to consider in performing an evaporation, all of which affect the rate of evaporation. Further, evaporating liquids experimentally can be time consuming, particularly for less volatile liquids (e.g., heavy petroleum distillates) and, as a result, the database may be limited in the number of liquids or levels of evaporation that are included.

In this research, a previously developed mathematical model was further investigated for applications in fire debris analysis. Briefly, the model, which is based on first-order kinetics, is used to predict evaporation rate constants for each compound in the ignitable liquid as a function of retention index. For a given evaporation level, the rate constants are used to predict the fraction of each compound remaining. The fraction remaining is plotted versus retention index to generate a fraction remaining curve for a given evaporation level. This curve is multiplied by the chromatogram of the unevaporated liquid to generate the chromatogram corresponding to that evaporation level.

To test the application of the mathematical model, three petroleum distillates (spanning the light to heavy subclasses) and gasoline were analyzed in the unevaporated state by GC/MS using a 100% dimethylpolysiloxane stationary phase, which was necessary to allow calculation of retention indices. The model was applied to the chromatogram of each unevaporated liquid to generate chromatograms corresponding to evaporation levels of 25%, 50%, 75%, and 90% by mass. Each liquid was also evaporated experimentally to the same levels and analyzed by GC/MS in a similar manner. The chromatograms of the evaporated liquids generated using the model and those derived experimentally were compared using Pearson Product-Moment Correlation (PPMC) coefficients. These coefficients offer a pairwise comparison to assess similarity between the chromatograms, with coefficients greater than 0.8 indicating strong correlation.

For the petroleum distillates, PPMC coefficients indicated strong correlation between the mathematically generated chromatogram and the corresponding experimentally derived chromatogram; however, there was a slight decrease in PPMC coefficient as evaporation level increased. For example, PPMC coefficients between the experimental and mathematically generated kerosene chromatograms ranged from 0.9942±0.0005 at 26% evaporated by volume to 0.954±0.002 at 90% evaporated by volume.

For gasoline at the 25% evaporation level, PPMC coefficients indicated a strong correlation between the mathematically generated and experimentally derived chromatograms (0.9620±0.0014). The PPMC coefficients again decreased as evaporation level increased; however, in this case, the strength of the correlation also decreased, with a PPMC coefficient of 0.535±0.015 at the 90% evaporation level, which indicates only moderate correlation. The lower correlation occurred in part due to the mass of highly volatile compounds present in gasoline. These compounds eluted before and during the solvent front, meaning that they were not detected using the current GC/MS method. After taking these compounds into account, the correlation between the mathematically generated and experimentally derived chromatograms increased to 0.986±0.002 at the 90% evaporation level, indicating strong correlation. This presentation will describe the application of the model and indicate ways in which this model could be applied in fire debris analysis.

Mathematical Modeling, Evaporation, Fire Debris Analysis

B47 Characterization of Aluminum (Al) Powders in Explosives Utilizing Particle Micromorphometry

JenaMarie Baldaino, BS, 5012 Coachmans Carriage Terrace, Glen Allen, VA 23059; Danica Ommen, MS, South Dakota State University, Mathematics & Statistics, Box 2220, Brookings, SD 57007; Joshua Dettman, PhD, 2501 Investigation Parkway, Quantico, VA 22135; Raleigh Parrott II, 2501 Investigation Parkway, Quantico, VA 22135; Jack Hietpas, PhD, FBI-ORISE, 2501 Investigation Parkway, Quantico, VA 22135; and JoAnn Buscaglia, PhD, FBI Laboratory, CFSRU, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, attendees will better understand the forensic potential of automated particle micromorphometry to aid in making comparisons between questioned and known Al powders. In addition, the results from this study may help provide insight into the method of Al powder manufacturing.

This presentation will impact the forensic science community by demonstrating the application of aluminum particle micromorphometry as a quantitative method for the characterization and comparison of explosive evidence, which may also provide valuable lead identification for forensic investigations.

Al powders have a wide variety of legitimate uses, are widely available without significant regulatory constraints, and are commonly used in pyrotechnics and explosives as a fuel to increase the heat of explosion.[1,2] Al powders can be bought from manufacturers or can be made inexpensively at home using basic instructional manuals and videos.[3] Due to their ease of production, bomb-makers commonly mix Al powder with ammonium nitrate or RDX to gain larger detonations for the construction of Improvised Explosive Devices (IEDs).[3,4] Al particle size and shape influence how reactive the Al powder will be; smaller particle size and flatter particle shapes will lead to a higher reactivity.[1] This research investigates the potential of using particle micromorphometry to differentiate Al powders, with the goal of comparing Al samples (e.g., known versus unknown source samples) as well as provide insights into production methods.

This presentation builds on the results from a small pilot study that determined that differences in manufacturing methods of Al powder produced differences in particle micromorphometry. A significantly larger-scale study (greater number and variety of samples with more detailed sample pedigrees) is currently being performed. Al powder samples were obtained from legitimate industrial manufacturers, seized IEDs, and "in-house" production from Al flake-containing commercial spray paint and ground Al foil. To replicate amateur methods of Al powder production, Al foil was ground in a consumer-grade blender as well as in a small 6lb rotary dual-drum ball mill. To obtain uniform particle size for high-quality microscopical imaging, the Al powder samples were wet-sieved with disposable polyester mesh. Transmitted light microscope images (*n*1,600 images/sample) of the Al samples were acquired using an automated stage. Dimensional analysis was calibrated using a National Institute of Standards and Technology (NIST) -traceable stage micrometer; polystyrene spheres of 100µm, 50µm, and 10µm were used as secondary standards to assess linear calibration. Images were batch processed using commercial image analysis software and customized code. Each image was pre-filtered using a high-pass filter to enhance edge detection and converted to a binary image. Seventeen parameters were measured for each particle within the image field of view including: area, aspect ratio, perimeter, roundness, mean diameter, mean feret, radii (maximum and minimum distance from particle centroid to edge), radius ratio, box height, box width, and fractal dimension. The large multidimensional datasets (*n*90,000-210,000 particles/sample) were analyzed using an open-source statistical package; the results from the multivariate statistical methods will be presented. Initial statistical results from the pilot study, which included only 23 Al powders, showed classification success rates ranging from 81%-94%, depending on particle size range.

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Aluminum Powder, Particle Micromorphometry, Automated Stage Analysis

B48 Identification and Separation of Nitrate Esters Using Both Liquid Injection Gas Chromatography/Mass Spectrometry (GC/MS) and Total Vaporization Solid Phase Microextraction (TV-SPME) GC/MS

Jordan Ash, BA, 1435 Stadium Way, Apt 2101, Indianapolis, IN 46202; and John V. Goodpaster, PhD, FIS Program, IUPUI, 402 N Blackford Street, LD 326, Indianapolis, IN 46202*

After attending this presentation, attendees will understand the value and need for a singular method that allows for the identification and separation of various nitrate esters found on exploded devices. TV-SPME/GC/MS is a new technique that has several benefits over traditional liquid injection.

This presentation will impact the forensic science community by providing a singular method of analysis for nitrate esters, saving both time and money as well as increasing sensitivity.

Given that most laboratories utilize a liquid injection procedure, a method has been developed using this technique; however, a novel technique based upon TV-SPME/GC/MS has also been used with great success in identifying compounds of interest. This novel technique has the inherent benefit of having a much lower limit of detection and can therefore allow for identification with less sample preparation and small sample sizes. This is of great interest as the explosive used in a device can be determined from smaller samples recovered from a post-blast site.

The detection and identification of post-blast residues is an important part of an explosives investigation; however, various methods must be used in determining the type of explosive that was used in the device. This study sought to determine if a single method could be found to separate and identify the various nitrate ester explosives as well as their degradation products. It was found that GC/MS could be used to differentiate a number nitrate esters such as Nitroglycerin (NG), Ethylene Glycol Dinitrate (EGDN), Pentaerythritol Tetranitrate (PETN), Erythritol Tetranitrate (ETN), Pentaerythritol Trinitrate (PETriN), 1-nitroglycerin, 2-nitroglycerin, 1,2-dinitroglycerin, and 1,3- dinitroglycerin.

Nitrate esters fragment identically within a mass spectrometer; therefore, having a true separation using a GC program prior to entering the MS is vital. Several variables were investigated using liquid injection, such as inlet temperature, flow rate, and inlet temperature ramp speed. In addition to liquid injection, a novel method was developed using TV-SPME/GC/MS. TV-SPME is a process that is most akin to immersion SPME. In immersion SPME, a two-phased system is present between the liquid containing the analyte and the SPME fiber. In TV-SPME, heat is used to drive all of the analyte into the gaseous phase. By taking a small amount of liquid that can be totally vaporized into the gaseous phase, one is able to create a two-phase environment between the analyte in the gaseous phase and the SPME fiber. This has the benefit of increasing sensitivity and decreasing sample preparation. Throughout this study, the following parameters were optimized: fiber type, incubation temperature, and multi-stage inlet temperature ramp. This method was able to separate and identify PETN, EGDN, NG, and ETN and the detection limits for these compounds was as low as 50 parts-per-trillion. Post-blast debris from the initiation of PETN-based plastic explosives was also analyzed.

Nitrate Esters, Explosives, SPME

B49 High-Sensitivity Detection and Separation of High Explosives in Environmental Samples

Christopher M. Rollman, BS, George Washington University, 2100 Foxhall Road, NW, Somers Hall, L14, Washington, DC 20007; Karen A. Brensinger, MFS, George Washington University/U.S. Army Forensic Toxicology Drug Testing Lab, 2490 Wilson Street, Fort Meade, MD 20755; Christine Copper, PhD, United States Naval Academy, 572 Holloway Road, Annapolis, MD 21402; Ashton Genzman, BS, United States Naval Academy, 572 Holloway Road, Annapolis, MD 21402; Jacqueline Rine, BS, United States Naval Academy, 572 Holloway Road, Annapolis, MD 21402; Ira S. Lurie, PhD, George Washington University, Dept of Forensic Science, 2100 Foxhall Road, NW, Somers Hall, Lower Level, Washington, DC 20007; and Mehdi Moini, PhD, George Washington University, Dept of Forensic Sciences, 2100 Foxhall Road, NW, Washington, DC 20007*

The goal of this presentation is to discuss a new technique to detect high explosives in environmental samples using Micellar Electrokinetic Chromatography/Mass Spectrometry (MEKC/MS).

This presentation will impact the forensic science community by examining how forensic chemists can use this rapid and highly sensitive method to separate and identify high explosives in contaminated sand, soil, and water samples.

High explosives constitute the majority of modern military and industrial explosive applications. Because of their wide use, their environmental footprint is becoming an issue. Therefore, identification of high explosives in soil and water is important. Currently, Gas Chromatography/Mass Spectrometry (GC/MS) and High-Performance Liquid Chromatography/Mass Spectrometry (HPLC/MS) are the preferred techniques for the analysis of explosives, yet both have drawbacks. GC/MS is not suitable for the analysis of thermally labile compounds, which include some high explosives, while HPLC/MS lacks sensitivity due to low ionization efficiency of high explosives under negative ionization. Therefore, a selective and sensitive method for the separation and detection of high explosives is desirable. In collaboration with the United States Naval Academy, a novel MEKC/MS technique was developed for the detection of high explosives using a complexation reagent.

Analyses were performed using a Beckman Coulter ProteomeLab™ PA 800 sheathless Capillary Electrophoresis (CE) interfaced to a Thermo™ Orbitrap Elite™ high resolution MS using underivatized fused-silica capillaries (20µm I.D., ~100cm in length) with a porous tip. Electrospray voltage was 1.1kV and the mass spectrometer heated capillary was 150°C. Analyses were performed using a Perfluorooctanoic Acid (PFOA) ammonium salt as a background electrolyte. All samples were injected using pressure (1psi for 4s) and a separation voltage of 25kV was used. Compounds were detected in negative ion mode as a complex with PFOA. Explosive samples from the United States Army Chemical, Biological, Radiological, Nuclear, and Explosives (CBRNE) Analytical & Remediation Activity Mobile Expeditionary Laboratory have been obtained, extracted, and analyzed using this newly developed MEKC/MS method.

High explosives that formed complexes with PFOA included RDX, HMX, tetryl, and PETN. Also, amino-dinitrotoluene formed a complex with PFOA. Other nitroaromatics were detected as molecular ions. The five explosives which formed complexes with PFOA had detection limits in the high parts-per-billion range and linear calibration responses over two orders of magnitude. The technique was successfully applied to the quantitative analysis of high explosives in sand samples.

Using PFOA as a background electrolyte, high explosives from contaminated sand samples could be separated and detected with high sensitivity.

High Explosives, Environmental Samples, CE/MEKC/MS

B50 Application of a Linear-Targeted Approach in Multiplex Amplification of the Mitochondrial Genome

Maureen Hickman, MS, Western Carolina University, 111 Memorial Drive, NSB 231, Cullowhee, NC 28723; and Kelly Grisedale, PhD, Western Carolina University, 111 Memorial Drive, NSB 231, Cullowhee, NC 28723*

After attending this presentation, attendees will have a better understanding of a linear-targeted approach designed to increase template DNA in low copy samples. Moreover, implementation of the linear approach in multiplex amplification and Massively Parallel Sequencing (MPS) of the whole mitochondrial genome will be presented.

This presentation will impact the forensic science community by providing an avenue to maximize data recovery from low quality samples.

In cases where limited or degraded nuclear DNA precludes the use of capillary electrophoresis to obtain a reliable Short Tandem Repeat (STR) profile, forensic scientists often turn to the mitochondrial genome due to its higher copy number per cell. The emergence of MPS in forensic science has enhanced the capability to recover extensive sequence information, thereby increasing the power of discrimination in mitochondrial DNA (mtDNA). Furthermore, MPS provides the breadth and depth of coverage necessary to detect low-level heteroplasmic variants across the mitochondrial genome (mtGenome) with a relatively small initial DNA input.

Though mtDNA may afford a more viable option when sample quality is questionable, there are instances where DNA yield remains too low to deliver a reliable haplotype. Polymerase Chain Reaction (PCR) -based techniques have been successful in increasing DNA yield of low-copy number samples; however, due to the exponential nature of PCR, increasing PCR cycle numbers can exacerbate stochastic sampling effects, especially in diploid nuclear DNA.[1,2] One method suggested to help alleviate this problem is targeted non-exponential amplification.[3] A Single Strand Extension Assay (SSEA) seeks to boost copy number prior to traditional analysis by employing a linear-targeted amplification approach. Briefly, samples are divided and amplified separately in two reactions with either a forward or reverse primer. Similar to PCR, SSEA includes denaturation, annealing, and extension steps, but with either forward or reverse primer in the reaction, only one additional copy of the product is produced after each cycle. Following SSEA, products from each reaction are pooled and sufficient template DNA is available for subsequent PCR analysis. Due to its haploid nature, the mtGenome provides an optimal target for evaluating the utility of SSEA, as the stochastic effects associated with diploid genomes (e.g., heterozygote imbalance, allele drop-out) will be excluded.

The utility of SSEA on mtDNA was initially tested for the hypervariable region. Commercially available DNA (HL-60) and DNA isolated from telogen hairs were diluted to low-template levels and amplified in separate forward and reverse reactions. Amplification success was evaluated on the Agilent® 2100 Bioanalyzer and absence of primer dimer was confirmed. Forward and reverse products were then combined and subjected to a second round of traditional PCR, prepared for MPS using Nextera® XT kit, and run on Illumina® MiSeq®. Preliminary results indicate that performing SSEA on low-template samples prior to traditional PCR provides sufficient target DNA for MPS.

To further test the effectiveness of SSEA, a previously developed low-volume multiplex PCR assay that amplifies ~350bp-650bp fragments around the mtGenome was employed. The multiplex assay has successfully amplified telogen hair and bone samples with minimal DNA input (fewer than 1,500 copies) and average MPS coverage ranges from 11,530 to 23,239. To implement SSEA using the multiplex assay, primers from either the heavy or light strand were carefully selected and extension performed separately for each of three multiplex reactions. Heavy and light strand products were then combined and subjected to a second round of traditional PCR, prepared for MPS using Nextera® XT kit and run on Illumina® MiSeq®.

The SSEA combined with the low-volume multiplex assay provides a viable option for maximizing data recovery from low-template DNA samples. Future research will focus on applying SSEA to other types of forensically relevant samples as well as evaluating SSEA performance in nuclear DNA using MPS.

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Linear Amplification, Mitochondrial Genome, Multiplex Amplification

B51 Assessment of Low-Level Error in Massively Parallel Sequencing (MPS) Data Sets Generated Using the Illumina® MiSeq® Platform and Synthesized Human Mitochondrial DNA Oligonucleotides

Brittania J. Bintz, MSc, 111 Memorial Drive, NSB 231, Cullowhee, NC 28723; Timothy Driscoll, PhD, West Virginia University, Dept of Biology, Life Sciences Building, PO Box 6057, Morgantown, WV 26506; and Mark R. Wilson, PhD, Western Carolina University, Dept of Chemistry/Physics, Forensic Science, Cullowhee, NC 28723*

After attending this presentation, attendees will better understand ongoing validation studies designed to assess the accuracy, precision, and reproducibility of the Illumina® MiSeq® sample preparation workflow and sequencing chemistry in human mitochondrial DNA (mtDNA) analysis.

This presentation will impact the forensic science by illustrating that the use of MPS technologies in forensic casework is eminent. Therefore, dissemination of information related to MPS validation studies is an important step in showing general acceptance of the method by the scientific community and, ultimately, the courts.

MPS sequencing methods are proving to be particularly well-suited for mtDNA analysis and may provide forensic analysts with a powerful tool that enables deconvolution of mtDNA mixtures or accurate quantitation of low-level heteroplasmy; however, some effort remains in validating the systems for such analyses.[1-5] Several MPS platforms are commercially available, each with a unique library preparation strategy and sequencing chemistry that may give rise to method-specific errors. Furthermore, since many alignment and variant-calling algorithms are available, there is limited consistency in the use of data analysis methods employed. Finally, no studies have been performed to determine what depth of coverage is required to confidently call a true biological low-level variant above the level of method-generated noise.

This study seeks to identify error rates associated with each step in the Illumina® MiSeq® MPS workflow. Initially, synthetic oligonucleotides with sequences matching the revised Cambridge Reference Sequence (rCRS) Hypervariable (HV) regions I and II of the human mtDNA genome were purchased from Life Technologies™. Each oligonucleotide was designed to contain Illumina® sequencing primers, flow cell adapters, and multiplexing indices on either end to enable direct sequencing without additional preparation. The oligonucleotides were also designed to contain restriction enzyme cut sites between the target sequence and Illumina® modifications. This design allowed for removal of Illumina® modifications so the same sample could be prepared for sequencing using the recommended library preparation strategies. Each synthetic oligonucleotide was sequenced: (1) directly with no additional preparation; (2) after Illumina® Nextera® XT library preparation; and, (3) after triplicate PCR amplification with target-specific primers followed by Nextera® XT library preparation. Samples prepared with treatments B and C were sequenced in duplicate to enable assessment of intra-run variation. Sequences were generated on the Illumina® MiSeq® with a v2 300-cycle run kit. Resulting sequence data was aligned to the rCRS using BWA-MEM alignment algorithm.[6] Variant calling was performed with SAMtools 0.1.19 using the consensus-caller and a maximum depth of 1,000.[7]

Error rates obtained from all sample treatments were compared to identify differences at each step in the library preparation workflow. Overall, data quality decreases as the sequencing progresses with late cycle basecalls being lower in quality than early cycle calls. Additionally, reverse reads tend to have lower average quality scores than forward reads. Overall, there does not appear to be a discernable effect from sequencing chemistry or PCR amplification during sample preparation. Ultimately, this experimentation sets the groundwork for validation of the Illumina® MiSeq® MPS system for mtDNA analysis in forensic casework.

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B52 Evaluation of Collection Protocols for the Recovery of Biological Samples From Crime Scenes

Dina Al Oraer, BS, UCLAN, 60 Victoria Mansions, Navigation Way, Preston, Lancashire PR2 2YY, UNITED KINGDOM*

After attending this presentation, attendees will understand how to improve the efficacy of the processes of collection and storage up to the point where the evidential material is received at a laboratory. Included in this presentation will be the effect of the environmental factors on degradation of collected samples before they reach the laboratory.

This presentation will impact the forensic science community by providing a clear indication of best practices in post-collection sample handling while in transit to the laboratory and may form the basis of future sample handling protocols.

The main focus in forensic genetics in the past 20 years has been to increase efficiency of the extraction and identification of DNA from a wide variety of evidence and to improve DNA profiling technology by making it more sensitive and robust. Much effort has been put into the improvement of DNA extraction and analysis techniques. Whichever technology is used, the precursors to extraction and analysis are the sample collection, handling, and storage. Collection, preservation, and storage of DNA are critical factors in ensuring reliable forensic genetics. These steps can have a fundamental impact on the quality of the sample and the resultant DNA profile; however, the methods used to recover DNA evidence from crime scenes have seen little development. This study proposed to improve the efficacy of the collection and storage up to the point where the evidential material is received at a laboratory.

Sample collection is one of the most critical steps in DNA profiling. Great care has to be taken to avoid contamination and degradation of the samples and the consequential spoiling of evidence. With the correct protocols, sample preservation in the laboratory can be carefully controlled, but this is more difficult in the field or when transporting from the scene of the crime to the laboratory. The preservation of the evidence is increasingly important when the environmental conditions are extreme and the time between collection and receipt by the laboratory is extended.

The collection of biological evidence with swabs using ultrapure water as a wetting agent was compared to the use of a propriety detergent-based wetting agent. The recovery of biological material using the detergent-based wetting agent is only marginally better than ultrapure water, but the post-collection stability is greatly improved. DNA degradation can be seen after approximately 6h at room temperature when using ultrapure water as the wetting agent. The detergent-based solution stabilized DNA for up to 48h, even when the temperature is increased to 37°C. The impact of this is likely to be limited in circumstances in which crime scene evidence can be kept at low temperatures until it reaches the laboratory; however, in contexts where this is problematic, the modified method for collection could have a large impact on the preservation of forensic evidence before it reaches the laboratory. The result of this initial research confirms that post-collection environmental factors have a significant impact on DNA recovery rates. Further research will be required to confirm and extend these results. Other substrates may be considered. This research should give a clear indication of best practices in post-collection sample handling while in transit to the laboratory and may form the basis of future sample handling protocols.

Forensic Genetics, Biological Samples, DNA

B53 Single Molecule Forensic DNA Characterization With Laser-Induced Nanopore Heating

Sarah J. Seashols Williams, PhD, Virginia Commonwealth University, Dept of Forensic Science, PO Box 843079, Richmond, VA 23284-3079; Christopher Angevine, BS, Virginia Commonwealth University, Dept of Physics, 701 W Grace Street, Box 842000, Richmond, VA 23284-2000; Nicole Auka, Virginia Commonwealth University, Dept of Forensic Science, 1015 Floyd Avenue, Box 843079, Richmond, VA 23284-3079; and Joseph E. Reiner, PhD, Virginia Commonwealth University, Dept of Physics, 701 W Grace Street, Box 842000, Richmond, VA 23284-2000*

After attending this presentation, attendees will recognize that pre-analysis sample characterization could be a useful tool in the forensic workflow. Attendees will be briefed on a novel method of DNA analysis using nanopore sensing and its potential applications to forensic science.

This presentation will impact the forensic science community by proposing a novel method for pre-analysis mixture deconvolution and promote more research toward that goal.

Forensic DNA analysis relies on techniques that can yield incomplete or very complex results, based on the number of people contributing to the sample. This creates a need to develop rapid screening methods that provide previews of the genotype(s) in the sample, especially given the impending costly and time-intensive high-throughput sequencing revolution of forensic human identification. Determining the number of amplified fragments in solution from a single amplified Short Tandem Repeat (STR) locus can indicate the number of individuals contributing to the evidence. A new approach has been identified that could provide this "preview genotype" of the sample, utilizing laser heating in conjunction with nanopore-based resistive pulse sensing to identify the number of differently sized DNA fragments in a given sample.

Nanopore sensing is a well-established technique that utilizes the Coulter-counting principle to detect single molecules. Briefly, an applied transmembrane voltage drives ionic current through an isolated pore and individual DNA molecules are driven into the pore, thus reducing the flow of current and giving rise to short-lived current blockades. The magnitude and duration of these blockades can be analyzed to infer information about the size of the DNA molecules. Nanopore-DNA studies showed that the time for a single stranded (ssDNA) molecule to move through an alpha hemolysin pore scales linearly with the number of bases. Given the short time that ssDNA spends moving through the pore (»2-3µs/base), it is difficult to distinguish between different, but similarly sized, DNA molecules.

This presentation will describe the efforts taken to address this issue through modification of the nanopore sensor with the addition of infrared laser heating and the study of double stranded DNA (dsDNA). Studies showed that heating the solution in and around a pore facilitates unzipping of the dsDNA and this process yields DNA pore transit times that scale exponentially with the length of the DNA molecules. This phenomenon was consistent over hundreds of replications. This suggests a way to clearly distinguish between current blockades from different-sized molecules, which could lead to a rapid and accurate technique for prioritizing samples for further forensic analysis. The results show that heat-induced residence time discrimination was enhanced over a range of DNA sizes up to 70nt. This discrimination also enables the relative proportion of different-sized DNA fragments in a binary mixture to be distinguished. Future efforts will expand these results to DNA sizes on the order of the commonly used forensic STR "mini" loci (60-100 nucleotides) with the goal of demonstrating mixture ratio analysis on samples containing alleles differing in size by 4nt-8nt.

Mixture Deconvolution, Nanopore, Forensic DNA

B54 Forensic Application of Massively Parallel Sequencing (MPS) With the Ion Torrent™ Multiplex Mitochondrial Genome Panel and Hi-Q™ Sequencing Chemistry

Jennifer D. Churchill, PhD, UNTHSC, 3500 Camp Bowie Boulevard, CBH-250, Fort Worth, TX 76107; Jonathan King, MS, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107; Joseph P. Chang, BS, Thermo Fisher Scientific, 180 Oyster Point Boulevard, South San Francisco, CA 94080; Sharon C. Wootton, PhD, 180 Oyster Point Boulevard, South San Francisco, CA 94080; Chien-Wei Chang, PhD, Thermofisher Scientific, 180 Oyster Point Boulevard, South San Francisco, CA 94080; Robert Lagacé, BS, Thermo Fisher Scientific, 180 Oyster Point Boulevard, South San Francisco, CA 94080; and Bruce Budowle, PhD, UNT Health Science Center, Forensic & Investigative Gen, 3500 Camp Bowie Boulevard, EAD 310, Fort Worth, TX 76107*

After attending this presentation, attendees will better understand a variety of the latest developments used in MPS on the Ion PGM™ platform such as the introduction of a new sequencing chemistry and the creation of a new forensically relevant marker system.

This presentation will impact the forensic science community by providing information and current progress on MPS that will support its eventual transition into forensic laboratories.

While still considered the "gold standard" of DNA typing in forensic laboratories, Capillary Electrophoresis (CE) -based technologies face limitations in scalability and throughput. Due to these limitations, sequencing beyond hypervariable regions I and II of the mitochondrial genome is rarely attempted in forensic laboratories. Advancements and developments in MPS technologies offer a strong alternative to the CE-based process, and the Ion PGM™ provides a promising MPS platform for forensic analysis with a read length, sensitivity of detection, and throughput that should incorporate well into forensic laboratories; however, transition of MPS technologies into forensic laboratories requires an efficient workflow, sequencing chemistry that produces robust and accurate data, and forensically relevant marker systems.

The Ion Torrent™ Hi-Q™ sequencing chemistry was evaluated to determine whether it had an effect on sequence quality. The whole mitochondrial genome of 31 individuals was sequenced with the original and new Hi-Q™ sequencing chemistries. Haplotype calls, coverage, strand balance, noise, and sequence quality through homopolymeric regions (i.e., deletion ratios) were evaluated for both data sets. Results were concordant between the sequencing chemistries for haplotype calls, coverage, strand balance, and noise level results; however, the Hi-Q™ sequencing chemistry showed an improved ability to sequence through homopolymeric regions, which was illustrated by a decrease in deletion ratios through these regions compared with the original sequencing chemistry.

Additionally, using the new Hi-Q™ sequencing chemistry, a comprehensive multiplex short amplicon panel was developed that spans the entire mitochondrial genome. This panel is currently comprised of two multiplexes with 81 primer pairs each that generate amplicons ≤175bps in length, making it well-suited for analysis of challenged samples. When used with the Ion Chef™, the workflow is sufficiently robust to support analysis of the entire mitochondrial genome. Thirty-one individuals were sequenced with this mitochondrial multiplex panel and sequence concordance, coverage, and strand balance were used to evaluate the quality and reliability of the data produced. Analysis showed that haplotype calls for these samples were concordant with whole mitochondrial genome data generated by long Polymerase Chain Reaction (PCR), coverage ranged from 307X to 8,583X, and strand balance illustrated reads were generated from both strands of the DNA. These results indicate robust and accurate data were generated. A serial dilution was completed for three individuals by varying the amount of input DNA from 1ng to 1pg and results illustrated the sensitivity of detection of this mitochondrial multiplex panel. Finally, successful analysis of historical skeletal remains with this mitochondrial multiplex panel demonstrated the great potential this panel offers for analysis of challenged samples. Overall, the quality of the data generated in this study supports the promising potential for incorporating whole genome mitochondrial analysis on the Ion PGM™ system.

Massively Parallel Sequencing, Forensic DNA Typing, Mitochondrial DNA

B55 From Fragment Isolation to DNA Amplification: A Detailed Protocol for Using Plant and Insect Material in Forensics

Megan L. Jackson, BS, 1 Joplin Court, Stafford, VA 22554; Kelly A. Meiklejohn, PhD, ORISE/FBI Laboratory, 2501 Investigation Parkway, Quantico, VA 22204; Jack Hietpas, PhD, FBI-ORISE, 2501 Investigation Parkway, Quantico, VA 22135; Libby A. Stern, PhD, FBI Laboratory - CFSRU, 2501 Investigation Parkway, Quantico, VA 22135; and James M. Robertson, PhD, CFSRU, FBI Laboratory, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, attendees will be aware of a tested protocol for the isolation, extraction, and amplification of DNA from soil biological fragments.

This presentation will impact the forensic science community by providing an alternative to bulk meta-genomics for the analysis of biological fragments recovered in soil samples.

In the current era of massively parallel sequencing, there has been an influx of studies documenting the biological diversity in soil samples using a bulk metagenomic approach. Insect and plant fragments have been collected for hundreds of years and more recently documented based on their geographic region by the National Bureau of Agriculturally Important Insects and the United States Department of Agriculture. Within a collected sample, there can be substantial diversity in the biological fragments. In most cases, it is not possible to classify them using morphology for taxonomic identification; however, by using conserved primers targeted to specific groups of organisms such as insects, plants, fungi, and bacteria, individual taxa can be amplified and sequenced to provide a detailed picture of the biological community. Although a metagenomic approach facilitates the collection of large amounts of data from small and even very degraded samples, the current usefulness of this technique mainly lies with cross-sample comparisons; the Operational Taxonomic Units (OTUs) identified are compared among samples to determine whether it is likely they came from the same source/location. Species-level taxonomic identification is not possible using this approach, but such data could prove useful for soil geoattribution considering biological organisms only inhabit specific ecosystems.

A molecular-based approach may provide a more accurate and reliable tool for species identification. One commonly used technique for molecular identification is DNA barcoding. DNA barcoding is based on the notion that there is substantial variation at the DNA level among organisms to permit discrimination (i.e., each species on Earth will have its own unique barcode sequence). As DNA is embedded in every cell, a DNA barcode approach can be used to identify insect and plant fragments found within a soil sample. The current project was focused on developing a protocol to successfully isolate DNA from *individual* biological fragments commonly recovered from soil samples. To facilitate taxonomic identification of the samples, the appropriate DNA barcoding region was amplified according to established protocols of expert researchers in the field.[1]

This presentation will outline the details of the protocol including: (1) fragment isolation, washing, and documentation (photography and weighing); (2) extraction of the total genomic DNA; (3) determination of the DNA quality in each sample; (4) Polymerase Chain Reaction (PCR) optimization for the amplification of the insect (*CO1*) and plant (*matK* and *rbcL*) barcoding regions from a broad range of samples; and, (5) techniques used to screen PCR amplicons prior to sequencing.

Reference(s):

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Individual Biological Fragment, DNA Extraction, PCR

B56 Investigation of the Genomics of Cannabinoid Biosynthesis in *Cannabis Sativa*

Robert W. Allen, PhD, Oklahoma State University, Center for Health Sciences, 1111 W 17th Street, Tulsa, OK 74107-1898; Lindsey N. Allen, BS, Oklahoma State University, Center for Health Sciences, 1111 W 17th Street, Tulsa, OK 74107; Jane Ketner Pritchard, BS, 1111 W 17th Street, Tulsa, OK 74107; Jun Fu, PhD, 1111 W 17th Street, Tulsa, OK 74107; Rachel Wellendorf, BS, Oklahoma State University, 1111 W 17th Street, Tulsa, OK 74107; and Lindsey Yoder, MSFS, Oklahoma State University, 1111 W 17th Street, Tulsa, OK 74107*

After attending this presentation, attendees will: (1) be introduced to the biosynthetic pathway leading to the production of cannabinoids; (2) correlate the biosynthesis of cannabinoids with the underlying molecular characteristics of the genes encoding the enzymes that synthesize cannabinoids; and, (3) relate the molecular genetics of enzymes involved with cannabinoid biosynthesis with the potential to manipulate this species genetically to produce cannabinoids with medicinal properties.

This presentation will impact the forensic science community by contributing to a newer understanding of how variability at the DNA level in marijuana can significantly impact the chemical composition of this plant that holds promise as a source of natural medicines.

Currently, there are 23 states and the District of Columbia that have legalized the medical use of marijuana and its chemical products.[1] In addition, four states and the District of Columbia have legalized the recreational use of the plant as well.[1] The increasing legal use of marijuana and its products has stimulated research into the pharmacological benefits of the principal chemicals of interest produced by cannabis strains and also the biosynthesis of these compounds known collectively as cannabinoids. Two principal cannabinoids, delta-9-Tetrahydrocannabinol (THC) and Cannabidiol (CBD) have received the greatest attention to date; however, more than 60 cannabinoid compounds have been identified in cannabis.[2] Paralleling interest in the chemical composition of cannabis has been an interest in the genetics/genomics of the plant.[3]

The research discussed here has concentrated on two of the biosynthetic enzymes involved in the production of two of the principal cannabinoids with known medicinal effect, THC and CBD. The enzymes THC synthase and CBD synthase, respectively, are responsible for the conversion of Cannabigerolic (CBG) acid into the acidic forms of THC or CBD, which when heated, spontaneously become decarboxylated to the mature drug forms. The genes encoding THC synthase and CBD synthase are more than 80% homologous in sequence and exist in the genome as intronless genes of 1,635 basepairs.[4]

The THC and CBD synthase genes present in numerous seized cannabis samples were subjected to next generation sequencing on an Ion Torrent™ platform. Findings from these studies have indicated that the THC synthase and CBD synthase genes exist in cannabis in active and inactive forms based upon the Single Nucleotide Polymorphisms (SNPs) present within the coding sequence. In the case of the THC synthase gene, 30 SNPs have been detected within the 1,635bp coding sequence and behave as a "haplotype." In other words, these SNPs travel together as a unit in different plants. It has been reported that some THC synthase genes are inactive as a result of SNP mutations that create stop codons or alter the amino acid sequence significantly. In hemp (which does not synthesize THC), this inactive haplotype is carried by both chromosomes, whereas plants producing THC genotype are either heterozygous for the inactive haplotype or homozygous for the active haplotype. Sequencing results showed that for CBD synthase, there were as many as 90 SNPs detected in the coding sequence for the gene. Future work involves obtaining additional plant materials rich in CBD to distinguish between the active and inactive haplotypes for the CBD synthase gene. It has been observed that seized marijuana may not exhibit appreciable amounts of CBD as would be expected from marijuana produced for illicit use even though it contained 3%-10% (by weight) THC.

Reference(s):

1. Cannabis in the United States, https://en.wikipedia.org/wiki/Cannabis_in_the_United_States
2. Brenneisen R. Chemistry and Analysis of Phytocannabinoids and Other Cannabis constituents. *Forensic Science and Medicine: Marijuana and the Cannabinoids*. Edited by: M.A. ElSohly © 2007, Humana Press Inc., Totowa, New Jersey.
3. van Bake H., Stout J.M., Cote A.G., Tallon C.M., Sharpe A.G., Hughes T.R., Page, J.E. The draft genome and transcriptome of Cannabis sativa. *Genome Biology* 2011, 12:R102 doi:10.1186/gb-2011-12-10-r102
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Cannabis Sativa, **Synthase Enzymes, Cannabinoids**

B57 Current Efforts on Developmental Aspects of Forensic Botany in Brazil

Renato T. Ferreira de Paranaiba, BA, Brazilian Federal Police, Lab. de Genética Forense, INC, Polícia Federal, SAIS Quadra 07 Lote 23, Brasília, Distrito Federal 70610200, BRAZIL; Carlos B. Carvalho, PhD, Sqs 212 Bloco K Apt 207, Brasilia, Distrito Federal 70275110, BRAZIL; Jorge Freitas, PhD, Departamento de Polícia Federal, Laboratório de Genética Forense, SAIS Quadra 07 Lote 23,, Brasília 70610200, BRAZIL; Gustavo Chemale, PhD, Departamento de Polícia Federal, Laboratório de Genética Forense, SAIS Quadra 07 Lote 23, Brasília 70610200, BRAZIL; and Katia Michelin, MSc, Departamento De Polícia Federal, Laboratório De Genética Forense, Sais Quadra 07 Lote 23, Brasília 70610200, BRAZIL*

After attending this presentation, attendees will better understand the current efforts of a forensic science subject that is less-developed, particularly in Brazil.

This presentation will impact the forensic science community by presenting the singularities of the forensic botany cases which will be discussed.

Botany is an area of study somewhat less developed than other forensic sciences. In fact, it can be said that botany is a promising subject of applied studies in criminal investigation. Plant DNA barcoding has emerged as a powerful and reliable tool for routine applications in forensic science. Furthermore, its application to plant species identification has provoked a renaissance for the use of taxonomy in basic science research.

Despite relevant initiatives and promised efforts by some groups, forensic botany in Brazil remains in its infancy when considering the advances of DNA technology. This is particularly surprising when the vast biodiversity of Brazil is considered; however, the application of plant DNA testing would have an impact beyond Brazil, as there are cases of plant materials suspected to be illicit that find their way into other countries. It would be useful to have a method to identify the same species in different places in an attempt to determine their origins. Demands addressed to forensic botany remain mostly unsolved; perhaps DNA testing could be helpful.

Cannabis sativa, *Salvia divinorum*, and *Lophophora williansii* are all classified as controlled plants under Brazilian legislation and all are prohibited. Punishment for trafficking may be a sentence of up to 15 years in prison.

Identification of these plant materials is conducted using morphological observation based on specific features in addition to chemical tests to identify the presence of specific compounds. More recently, the intrinsic limitations of these techniques are giving rise to the opportunity of using DNA analysis, especially due to the advantages of this technology.

Seeds or leaves from plant material were pulverized and DNA was extracted using commercial kits. Universal Polymerase Chain Reaction (PCR) primers were used that would amplify four DNA regions (*matK*, *rbcL*, Intergenic Spacer (IGS)_trnL-trnF, and Internal Transcribed Spacer (ITS)) for most land plants. PCR amplifications of the DNA were performed in a 9700 Applied Biosystems® thermal cycler. PCR products were checked using a 1% agarose gel electrophoresis and ultraviolet detection by spectrophotometer and purified with the Exonuclease I and Shrimp Alkaline Phosphatase (SAP). Sequencing of the PCR products was performed using both the forward and reverse primers in PCR amplification and the BigDye Terminator 1.1 kit. After purification, the cycle sequencing products were detected by an Applied Biosystems® 3130 Genetic Analyser with Data Collection and Sequencing Analysis software. Sequences were assembled and evaluated with the SeqScape® software and were compared to those present in on-line databanks (Genbank® and Barcode Of Life Data Systems (BOLD) v3). Further analysis, including phylogenetic trees, was performed on Mega 6 software.

The kingdom Plantae is a very complex universe. Forensic botany is taking its earliest yet very important steps and its development in Brazil is even more recent than in other countries. The first experiences of a Brazilian forensic DNA laboratory with forensic botany are reported here; three cases regarding this matter are described along with their key points. Finally, an interesting point regarding the DNA extracting method is discussed. Plant DNA barcoding may fit well in the automated technology already available at many forensic laboratories. Adding plant DNA testing may be easier based on past experience with implementing techniques for forensic human DNA.

Plant, DNA, Barcoding

B58 Inferring Geographical Origin of Forensic Evidence Via DNA Barcodes

Jack N. Lane, MS, Bode Technology, 10430 Furnace Road, Ste 107, Lorton, VA 22079; Michael N. Parsons, MS, 10430 Furnace Road, Ste 107, Lorton, VA 22079; and Donia Slack, MS, 10430 Furnace Road, Ste 107, Lorton, VA 22079*

After attending this presentation, attendees will understand the potential applications of using the DNA signature of regional plants for investigative leads and the ability to attribute plant material to a point of origin. Modern databases and sequencing technologies allow for increasingly more accurate determination of the geographical origin of plant material when only a minimal amount of plant debris may be available.

This presentation will impact the forensic science community by offering new insight into the effectiveness of plant barcoding for a forensic application of attributing a region of interest to potential evidence. Attendees will also better understand the performance of specific barcoding genes in various geographical areas.

During a forensic investigation, a wide variety of evidence types may be presented to an investigator, each offering a unique element or perspective to a crime. One particular type of evidence that may often be underutilized during investigation is plant debris. Plant material, when exploited for forensic leads, can assist in generating source attribution of evidence matter, resulting in inference of location and/or routes of travel. Forensic botany has traditionally relied on identification through morphological assessment of plant material. These methods often require well-preserved plant specimens or multiple portions of a plant that may not be available to the investigator. To overcome the limitations of traditional plant identification methods, it was proposed that sequence analysis of the widely accepted plant barcoding gene for ribulose bisphosphate carboxylase (*rbcL*) be used to identify plant specimens from mock evidentiary samples. Mock evidentiary samples were prepared using morphologically identified plant material collected from three specific locations in defined areas of the east coast of the United States: the Northeast, Mid-Atlantic, and Southeast regions. After collection, specimen DNA was extracted using the MoBio PowerPlant® Pro DNA extraction kit. The resulting mixture was sequenced on the Roche® 454 GS Junior next generation DNA sequencing platform. Using the CLC Genomics software, unique sequences were identified and used to query two existing plant barcode databases including Genbank® and Barcode Of Life System (BOLD). The degree of success was determined by assessing the accuracy of the range of distribution to the known origin of the mock evidentiary sample's constituents. By exploiting these ubiquitously used plant databases, this technique bypasses the need for expensive and laborious *de novo* databasing of plant genomes for a specific forensic application.

Results of the study indicate that *rbcL* universal primers can be utilized on the Roche® 454 GS Junior to sequence plant mixture samples. The geographical origins for two of the three collection regions were successfully identified using heat maps that illustrate the presence of each identified plant specimen within each collection region. Although the heat maps demonstrated a fair amount of overlap, the Northeast and Southeast regions showed clear delineations of attribution which allowed for the plant material to be sourced back to a corresponding point of origin. The Mid-Atlantic region could not be attributed to a plant mixture sample, as the sample's constituents generated vast native geographical ranges that could not confidently be attributed to a particular area of isolation. Thus, this research demonstrated that successful and confident source attribution of evidentiary plant debris is strongly reliant on the genetic variation of the sample and the representation of plants with a limited geographical distribution. It is important to note that the data reflect the success rate of only a single proposed barcoding gene, *rbcL*. Therefore, this technique may better serve the forensic community if supplementary barcoding genes are exploited to generate enhanced sequencing success and downstream plant identification.

Geosourcing, DNA Barcoding, Next Generation Sequencing

B59 Botanical DNA Evidence in a Case of Robbery and Property Crime: Application of High Resolution Melting Analysis of *Triticuma Aestivum* L. Grains

Alejandra Figueroa, BSc, Policia de Investigaciones de Chile, Aldunate 620, Temuco, CHILE; Jaime H. Solano, PhD, Universidad Católica De Temuco, Avenida Rudecindo Ortega 02950, Temuco 4813302, CHILE; Leonardo I. Anabalon, Universidad Católica De Temuco, Avenida Rudecindo Ortega 02950, Temuco 4813302, CHILE; and David A. Gangitano, PhD, Sam Houston State University, 13906 Paradise Valley Drive, Houston, TX 77069*

After attending this presentation, attendees will understand the importance of the use of genetic botanical evidence in casework.

This presentation will impact the forensic science community by increasing fundamental understanding of the application of real-time Polymerase Chain Reaction (PCR) technology and DNA barcoding to the discriminatory analysis of plants.

Forensic botany is an emerging discipline that has evolved rapidly during the past few years. Botanical evidence is usually found at crime scenes and sometimes is the only available element for criminal investigations in cases where other evidence is absent. Different molecular techniques have been applied to analyze botanical evidence including Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter-Simple Sequence Repeat Amplification (ISSRs), chloroplast and mitochondrial DNA, and Short Tandem Repeats (STRs). In recent years, research has been focused on DNA barcoding to determine the species of different organisms through sequencing of conserved DNA regions such as Cytochrome C Oxidase I (COI) or Internal Transcribed Spacer (ITS). Another application of DNA barcoding is the use of real-time PCR combined with High Resolution Melting (HRM) analysis to discriminate specific conserved DNA regions of closely related botanical species. For melting temperature (Tm) determination, an intercalating fluorescent dye is added to the real-time PCR reaction and a derivative melting curve is generated. Distinct nucleotide sequences of a conserved DNA region will provide different Tm.

A robbery and crime property case at a farm was investigated using real-time PCR combined with HRM analysis. After burning two yards of a wheat field, the suspects stole several tons of a brand new variety of wheat, transported it in a truck, then distributed it among people living in a nearby community. The truck was found by the police and wheat grains were collected from the vehicle.

Genetic analysis of wheat grains collected from the truck (evidence) was performed, and the results were compared to those obtained from wheat plants collected at the farm (crime scene). Control wheat grains were also included in the analysis. Briefly, wheat DNA was extracted from the grains using the Plant DNAzol® kit by Invitrogen™ and quantified with a fluorometer Qubit® 2.0 by Invitrogen™. HRM analysis using ITS 2-3 primers was performed in an Illumina's® Eco real-time PCR system. HRM analysis showed indistinguishable melting curves for wheat grains collected in the truck (evidence) and grains collected at the farm (crime scene).

The HRM strategy enabled the "molecular traceability" of the wheat grains to the crime scene, demonstrating the usefulness of this approach for the identification of closely related species and its application as a potential forensic molecular tool. Two suspects were arrested and prosecuted.

Forensic Botany, DNA Typing *Triticum Aestivum* **L, DNA Barcoding**

B60 Using DNA Barcoding to Detect Fish Substitutions in Brazil

Carlos B. Carvalho, PhD, Sqs 212 Bloco K Apt 207, Brasilia, Distrito Federal 70275110, BRAZIL*

After attending this presentation, attendees will understand DNA barcoding and the use of this technique in Brazil by a forensic laboratory to detect fish substitutions.

This presentation will impact the forensic science community by reinforcing the utility of DNA barcoding in forensic casework, including food fraud investigations.

Fish substitutions are usually a fraudulent way of making a product more profitable by swapping high-value species for lower-value ones. Besides being a form of economic deception, this illegal practice may cause health problems or damage the environment. The inadvertent consumption of some species that have the potential to cause allergic reactions, contain toxins and contaminants, or other species that are currently overexploited or protected, are some of the consequences of this illegal practice. Since processed fish products usually lack the external morphological characteristics used for species recognition, fraudulent substitutions are very common.

When morphological identification is compromised, genetic identification can be used to associate unknown samples to a reference sample by comparing sequences of mitochondrial genes. DNA barcoding is a universal system for cataloging and identifying species based on approximately 650 base pair sequences of the Cytochrome c Oxidase (COI) mitochondrial gene. Created as part of this system, there is an international publicly available reference database Barcode Of Life Data Systems (BOLD), with authenticated sequences and a search engine used for species identification. DNA barcoding has been used successfully to identify fish substitutions in many countries in the past few years.

In this study, the Brazilian Federal Police DNA Laboratory used DNA barcoding to identify 93 fish fillet samples collected from ten seafood processing plants in 2013 and 2014. The large majority (81%) of the samples were mislabeled. Among the substitutions, the laboratory found the exotic cheaper species of flatfish *Atheresthes stomias* and *Limanda aspera* sold as the Brazilian coast genus *Paralichthys*, the Alaska pollock *Gadus chalcogrammus* being passed off for the Argentine hake *Merluccius hubbsi*, and the protected Wreckfish *Polyprion americanus* labeled as a grouper species. Several other cases of substitutions involving species commonly traded in Brazilian markets are also discussed. Because of these identifications, some processing industries in the southern region of Brazil were put under special supervision by the Brazilian food safety authorities. This study represents a pioneer use of DNA barcoding in food fraud investigations by a police laboratory in Brazil and the results obtained reinforce the utility of this technique in forensic casework.

DNA Barcoding, Fish Substitutions, Food Fraud

B61 Effects of Bacterial DNA on Human Profiles

Kevin G. Smolar, MS, 1950 N Talbott Street, Apt 4, Indianapolis, IN 46202; Gina Dembinski, MS, Indianapolis, IN; and Christine J. Picard, PhD, 723 W Michigan Street, SL 306, Indianapolis, IN 46202*

After attending this presentation, attendees will understand the effects of bacterial DNA contamination on a human DNA profile, which not only affects quantitation results of the human sample but also produces artifacts that can be present in the resulting profile specific to the bacterial species tested.

This presentation will impact the forensic science community by demonstrating that bacterial contamination affects the sample quality and therefore the development and interpretation of a human DNA profile. For quantitation procedures, if there is a large presence of bacteria, they may skew the results of the human DNA present and can affect the quality of the development of the DNA profile. Furthermore, the presence of extraneous DNA on human DNA profiles often results in a reduced number of loci amplified, which in turn would affect interpretations and lower the statistical power of discrimination. As most kits are developed to be human specific, these results indicate that caution should still be used in interpreting the human DNA profile, especially in cases of decomposition in which there would be higher levels of bacterial contamination.

Most biological evidence obtained from crimes scenes is found in non-sterile environments, and therefore there is an opportunity for the forensic samples to become contaminated with environmental DNA. In particular, the effects of bacterial species that may contaminate forensic samples have not been extensively studied. This type of information could be especially important in cases where decomposition has occurred and bacteria are in abundance. The environmental effects on DNA samples via contamination from bacterial sources were examined by intentionally spiking human DNA samples with known concentrations of DNA from 16 common bacteria species associated with humans using the Quantifiler® Human DNA quantitation kit and the PowerPlex®16 HS system. Single species of bacterial DNA were added in varying quantities (1ng, 50ng, and 100ng) to a standard 1ng human DNA sample. Results indicate that as increasing amounts of bacterial DNA were added to quantitation reactions, the apparent amount of human DNA decreased, due to possible polymerase competition. During genotyping, *Bacillus subtilis* produced an artifact peak at the Thyroid Peroxidase (TPOX) gene locus near or at the "5" allele, even when only 1ng of bacterial DNA was present in the sample, and the same artifact was also still amplified when the bacterial DNA was tested alone, without human DNA present. With other bacterial species, there was severe allele and locus dropout, especially at D18S51, D16S539, and CSF1PO.

Bacteria, Decomposition, PowerPlex®16 HS

B62 Optimal Time for Forensic Screening of Evidence Based on Fluorescent Variation of Seminal Fluid

Jack N. Lane, MS, Bode Technology, 10430 Furnace Road, Ste 107, Lorton, VA 22079; and Donia Slack, MS, 10430 Furnace Road, Ste 107, Lorton, VA 22079*

After attending this presentation, attendees will understand the impact that both time and temperature have on human seminal fluid. Attendees will learn of the optimal detection window of time for seminal fluid based on the fluorescent trends observed.

This presentation will impact the forensic science community by providing an understanding of the natural variation of the fluorescent properties of seminal fluid as exposure time to the environment increases. Understanding the degree of variation in seminal fluid fluorescence over time may help investigators approach an investigation site or assist in the analysis of collected material. Providing investigators with a confident approach to seminal fluid detection will increase detection events while also assisting in scenarios in which no seminal fluid fluorescence is detected. The additional information gained from items processed using a detailed understanding of seminal fluorescence may aid in the conviction or exoneration of individuals associated with evidentiary items in which the presence or absence of seminal fluid served as a pivotal point of an investigation. The fluorescent trends observed may also help explain variation in findings of multiple laboratories or assist re-investigations as evidence may have aged following the collection or detection of the event in question.

During any collection scenario, detection of biological fluids such as seminal fluid on evidence is critical to its recovery. In the case of semen, detection is often dependent on analyst's ability to visualize the fluorescence that occurs naturally when seminal fluid is subjected to light in the wavelengths ranging from approximately 365nm to 480nm and visualized with an appropriate filter. Although it is well understood that seminal fluid can be viewed using an alternate light source due to the natural fluorescence of riboflavin and its cofactors flavin mononucleotide and flavin adenine dinucleotide, the degree to which human seminal fluid fluorescence may increase or decrease over time is not well documented. The present study explored the relationship of semen fluorescence over time to determine if an optimal detection window exists as well as the variability in semen fluorescence. To determine this detection window and assess the variability, multiple replicates of semen were collected from three donors and deposited into a 96-well optical plate. Samples were either left at room temperature 20°C-24°C or placed in a heated environment at 32°C. The samples were then excited at 365nm and emission was detected, in relative fluorescent values, at 465nm at multiple time intervals ranging from time 0hrs to 312hrs over the course of three weeks. Excitation and emission detection was performed using a standard 96-well fluorescent microplate reader, accessible to any forensic laboratory.

Results indicate that seminal fluid at time zero exhibits less fluorescence than aged or even heated seminal fluid. A statistically significant difference was observed for each of the three donors used when comparing the initial spotting values of seminal fluid and each of the succeeding time points. Temperature was also found to increase the rate at which fluorescent values increase; a statistically significant difference was observed between samples subjected to 32°C incubation and those incubated at room temperature, 20°C-24°C. Samples subjected to elevated temperatures produced higher fluorescent values than comparable samples held at room temperature. These results indicate that in high-profile cases in which detection of semen is critical to evidence collection, it may be preferable to allow an item of evidence to age at elevated temperatures, or even room temperature, before an analyst commences a forensic screening for semen using alternate light sources.

Semen, Alternate Light Sources, Seminal Fluid Fluorescence

B63 The Identification and Analysis of Burnt Bloodstains

Rebecca Nelson, BS, Eastern Washington University, Dept of Chemistry & Biochemistry, 226 Science Bldg, Cheney, WA 99004-2440; Maranda M. Hirst, 1707 E Liberty, Spokane, WA 99207; and Peter Bilous, PhD, Eastern Washington University, Chemistry & Biochemistry, 226 Science Bldg, Cheney, WA 99004-2440*

After attending this presentation, attendees will learn: (1) which color-based blood screening test is the most effective for the identification of bloodstains exposed to high temperatures; and, (2) which bloodstains are likely to yield informative DNA profiles.

This presentation will impact the forensic science community by improving the overall efficiency with which crime scene investigators and forensic scientists analyze bloodstains from crime scenes that have deliberately been set on fire by criminals in an attempt to destroy evidence.

Various color-based and light-emitting blood screening tests are used to locate and tentatively identify the presence of blood at crime scenes. Typically, only those stains which yield a positive result are collected for subsequent DNA analysis; however, when criminals attempt to destroy evidence using deliberately set fires, for example an arson-homicide case, blood screening tests may not be effective and potentially useful stains may be missed. Furthermore, high temperatures cause significant DNA degradation, thus affecting the quality of the resulting DNA profiles.

In this study, three color-based blood screening tests were compared for their ability to identify bloodstains that were exposed to a range of temperatures that may occur in a structural fire. Polymerase Chain Reaction/ Short Tandem Repeat (PCR/ STR) analysis was conducted using the AmpFℓSTR® Profiler Plus® DNA typing kit to determine the impact of heat exposure on the resulting DNA profiles.

The three color-based blood screening reagents used were O-Tolidine (OT), phenolphthalein (KM), and the tetramethylbenzidine-based Hemastix® test strip. To determine the limits of detection of each reagent, bloodstain smears were made on glass and tile surfaces using neat, 1:10, 1:100, or 1:1,000 dilutions of non-human blood. Bloodstains destined for DNA analysis were made on glass using a mixture of non-human blood and human buccal cells. Samples were exposed to temperatures ranging from 60°C to 630°C for 5, 10, or 15 minutes.

All three reagents worked equally well with bloodstains prepared from neat and 1:10 dilutions of blood for all of the test conditions except those which were in direct contact with the alcohol-based flame. Only OT gave positive results with the latter samples. With respect to latent bloodstains prepared from 1:100 and 1:1,000 dilutions of blood, Hemastix® outperformed both OT and KM reagents. None of the reagents gave positive results with latent bloodstains which were in direct contact with the flame.

Not surprisingly, time and temperature were critical factors affecting the degree of DNA degradation and the quality of the resulting DNA profiles. Samples located 15cm above the flame (~160°C) generated complete DNA profiles with all of the exposure times tested. Samples located 10cm above the flame (~250°C) generated full DNA profiles but only for the five-minute exposure time. No profiles were obtained with the 10- and 15-minute exposure times. For samples located 5cm above the flame (~380°C), only those stains exposed for five minutes generated DNA profiles, and only partial genetic information was obtained. Stains which were in direct contact with the flame (~630°C) for five minutes generated extremely limited genetic information.

In summary, the identification of blood is possible in cases where an incendiary fire was used to destroy bloodstain evidence. The three reagents were equally effective for the analysis of visible bloodstains; however, only OT was effective with stains which were in direct contact with the flame. Hemastix® was the best reagent to use with latent bloodstains. These results showed that useful DNA profiles can be obtained from most of the tested bloodstains. The quality of the DNA results depended on the temperature and duration of heat exposure.

Burnt Bloodstains, O-Tolidine, Hemastix®

B64 Migration of Seminal Fluid Components and Spermatozoa in Semen Stains Exposed to Moisture

Lyndsey T. Brown, BS, 135 Northbridge Drive, Mooresville, NC 28115; Robin W. Cotton, PhD, Boston University School of Medicine, Biomedical Forensic Sciences, 72 E Concord Street, R 806, Boston, MA 02118; and Amy N. Brodeur, MFS, Boston University School of Medicine, Biomedical Forensic Sciences, 72 E Concord Street, R806, Boston, MA 02118*

The goal of this presentation is to provide attendees with a general understanding of the observed migration patterns of seminal fluid components and spermatozoa when semen stains are exposed to varying amounts of water and allowed to dry while positioned at different angles.

This presentation will impact the forensic science community by suggesting modifications for processing sexual assault evidence that has been exposed to moisture in order to maximize the likelihood of detecting semen and spermatozoa.

Typically, semen testing involves presumptive and confirmatory tests to determine the region in which a semen stain has been deposited prior to initiating DNA analysis; however, previous research has shown that the soluble components of seminal fluid, but not spermatozoa, migrated from their original location on cotton cloth upon exposure to porcine decomposition fluids and rainfall/dew.[1] This indicates that preliminary testing and detection techniques may result in areas being sampled that will not yield a successful DNA profile. It is hypothesized that the more moisture a semen stain is exposed to, the greater distance the soluble seminal fluid components will travel, but that minimal spermatozoa migration will occur. It is also reasoned that the extent and direction of semen migration will be dependent on the degree of wetness and the position (angle) of the substrate at the time of moisture deposition.

Neat semen was deposited onto swatches from cotton sheets and allowed to dry before being sprayed with 2mL, 5mL, or 10mL of water. The swatches were allowed to dry while lying flat, at 45°, or at 90°. A total of 13 swatches were created: one at each moisture level at each angle and one control with no water applied. The swatches were viewed with an Alternate Light Source (ALS) at 450nm using orange barrier filter goggles and photographed. Three swatches were sprayed directly with Acid Phosphatase (AP) Spot reagent to determine any potential interference with subsequent P30 and DNA analysis as well as to help determine distances for sampling. Three-millimeter punches were taken from each swatch at 13 locations (one from the center of the stain and four at 1cm, 4cm, and 7cm out from the perimeter of the stain in multiple directions), and were extracted for two hours prior to testing for the presence of P30.

Observation of the swatches under ALS revealed that the fluorescing component(s) of semen traveled up to 3cm from the original stain location. Less migration of fluorescence was observed with the samples exposed to 2mL of water compared to the samples exposed to 5mL and 10mL, regardless of the angle. The samples exposed to moisture while positioned at an angle showed fluorescence migration primarily to the sides and below the stain as expected due to gravity. This effect was more pronounced in the samples at 90° compared to those at 45°.

AP testing showed positive results beyond the original stain region, demonstrating seminal fluid migration for several centimeters in all directions. Positive P30 results were obtained for all swatches at 1cm from the stain in at least one direction. The sample exposed to 10mL of water at 90° also displayed a positive P30 result at 4cm from the stain.

Microscopic examination of slides made from the extracts of each cotton punch revealed minimal spermatozoa migration for all swatches; the majority of the samples outside of the stain deposition area showed no spermatozoa, although a few showed a single sperm cell. These findings demonstrate that the soluble components of semen stains that often aid in detection will migrate when exposed to moisture, while sperm cells containing genetic material largely remain in their original location. Results of DNA testing on select areas from each swatch at varying distances will be presented.

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Semen Detection, Sperm, Moisture

B65 "Who Is My Father?" The Role of Forensic Genetics in The Resolution of a Paternity Case

Ciro Di Nunzio, MFS, PhD, Magna Graecia University, Viale Europa, Germaneto, Legal Medicine, Catanzaro 88100, ITALY; Isabella Aquila, MD*, Viale Europa, località Germaneto, Policlinico Universitario, S Venuta-Medicina Legale, Catanzaro 88100, ITALY; Michele Di Nunzio, BS, Università Magna Graecia, Viale Europa, Località Germaneto, Catanzaro, ITALY; Matteo Borrini, PhD, Liverpool John Moores University, RCEAP-School of Natural Science & Psych, Byrom Street, Liverpool L3 3AF, UNITED KINGDOM; Maurizio Saliva, MD, Via Carlo Maria Rosini 51, Pozzuoli 80078, ITALY; Flavio Saia, BS, Università Magna Graecia, Viale Europa, Località Germaneto, Catanzaro 88100, ITALY; and Pietrantonio Ricci, Viale Europa-Località Germaneto, Catanzaro, ITALY*

After attending this presentation, attendees will understand the problems associated with the identification of skeletal remains in southern Italian cemeteries in controversial parental relationship cases. The collaboration of experts in forensic pathology, anthropology, and genetics can greatly speed up the process of identifying the skeletal remains used in these cases.

This presentation will impact the forensic science community by suggesting a schematic approach in controversial parental relationship cases based on mixed skeletal remains.

In the Italian legal system, decedents must be buried in sealed wooden coffins and may be exhumed after a period of five to ten years. Each body is wrapped in a single sheet, inside of which is placed a document with brief details necessary for quick identification in the event of subsequent inspections. The number of bodies found in a tomb and information about the date of birth and death of each of these bodies are listed in the cemetery register.

A case of forensic judicial paternity is presented. An inquiry was made by a 64-year-old man (DG) against an alleged father (SLx). DG wanted to know who his biological father was. Unfortunately, DG learned that the alleged father had died. SLx was 72 years old at death, and his remains were placed in the family tomb with the remains of four other members of the family: the father (SP) (87 years old at time of death), the mother (88 years old at time of death), and two brothers (SF1-2) . At first, the judge had authorized the comparison of profiles between the presumed father (SLx) and the requesting party (DG); however, a preliminary inspection of the family tomb showed that cemetery personnel had not put the document with the critical information within each sheet. The document was placed only in the sheet that wrapped the remains of one the brothers of SLx. Therefore, the judicial authorities ordered a new investigation in order to establish through morphological and anthropometric assessments the identity of the skeletal remains in the tomb. Genetic testing was conducted on all human remains in order to obtain genetic profiles to be compared with the genetic profiles obtained from DG and his son (DGS). The forensic approach used was the one used to identify bodies following a mass disaster. At first, a consultation between the geneticist and the relatives of the decedents occured. This consultation provided very important information.

After a generic identification based on morphological and morphometric data, a radiological investigation was performed. Genetic analysis was conducted by comparing the genetic profiles obtained from the fragments of the femurs of SLx, SP, and SF with the profiles of DG and DGS. Genetic analysis showed the parental relationship between SP, SF, DG, and DGS, but no relationship was found with SLx with the remains that were assumed to belong to the alleged father. Therefore, the genetic puzzle was solved indirectly by comparing the genetic profiles of the relatives of the alleged father. The same genetic testing showed that during the displacement of the bones in the family tomb, the actual remains of SLx were lost and replaced with the remains of an unrelated individual. Moreover, DG learned that he was the grandson of SP and thus became aware of his origins and biological family despite the genetic investigation proving that the remains of the alleged father didn't exist and that SLx was unknown. This case of alleged paternity opened another court case about the true identity of the remains of SLx.

The multidisciplinary approach allows, in cases of doubt, for the identification of commingled human remains through the use of the same method used for mass disasters. The close collaboration between different disciplines such as forensic pathology, forensic anthropology, forensic radiology, and genetic forensic experts confirmed that these areas should always be used in paternity puzzles, especially when it comes to comparisons with buried human remains. The anthropological, radiological, and osteological evaluations can help in the identification and attribution of associated macroscopic remnants, while the genetic analysis, through the enhancement of the sampled subjects for molecular comparisons, can confirm the hypothesis of the investigators.

Forensic Science, Human Identification, DNA Paternity Testing

B66 Human Remains in Southern Italian Cemeteries: When the Type of Burial Influences the Results of DNA Extraction

Ciro Di Nunzio, MFS, PhD, Magna Graecia University, Viale Europa, Germaneto, Legal Medicine, Catanzaro 88100, ITALY; Isabella Aquila, MD*, Viale Europa, località Germaneto, Policlinico Universitario, S Venuta-Medicina Legale, Catanzaro 88100, ITALY; Maurizio Saliva, MD, Via Carlo Maria Rosini 51, Pozzuoli 80078, ITALY; Michele Di Nunzio, BS, Università Magna Graecia, Viale Europa, Località Germaneto, Catanzaro, ITALY; Francesco P. Busardo, MD, Viale Regina Elena 336, Rome, ITALY; Vittorio Fineschi, MD, PhD, University of Foggia, Forensic Pathology Dept, Ospedale Colonnello D'Avanzo, Foggia I-71100, ITALY; and Pietrantonio Ricci, Viale Europa-Località Germaneto, Catanzaro, ITALY*

After attending this presentation, attendees will better understand how the type of burial influences DNA extraction.

This presentation will impact the forensic science community by demonstrating the importance of the treatment of remains in southern Italian cemeteries and how this may promote the preservation of human bones and the success of DNA analysis.

Death causes the definitive transition from one social status to another. Understanding the nature of bodies and their status after death helps us appreciate this transition, which is encountered every time someone dies. Burial rituals are among the few visible forms of practice that may hint at beliefs about an afterlife. The practices, which include social, physical, body treatment, grave location, and cemetery organization, are determined by a complex mix of factors.

In the Sud Italian popular culture, prior to burial the deceased is in a transitional stage between life and death. After the 18th century, a double burial procedure was performed in order to verify this successful transition. In the first step, according to Presidential Decree 285/90, cemetery staff buried the corpse in a sealed wooden coffin. In the second step, after a period of five to ten years, the same staff exhumed the corpse. Family members washed and disinfected the remains, then wrapped them in a bed sheet and buried them in a stone niche for their final resting place.

Analyzed human remains were made available by judicial authorities for genetic identification or parental testing. The remains were in several decomposition stages due to different exposures to biotic and abiotic factors. The remains were collected from: (1) fire crime scenes where people killed by gun shots were burned to destroy evidence; (2) open spaces such as forests where missing people were found after a focused search; and, (3) cemetery areas were the remains were buried.

A correct analytical process is essential in order to perform DNA extraction from recovered human remains. Although DNA extraction can be carried out on any body part, compact femur bone represents the most suitable tissue, especially when DNA is degraded due to postmortem degenerative phenomena. The effects of decomposition on quality and concentration of DNA extracted from these mortal remains as well as the quality of obtained electropherograms were reviewed retrospectively on 100 femur fragments analyzed during the period of 2001 to 2014. The quality and quantity of extracted DNA, understandably, were often low, but the Short Tandem Repeat (STR) genetic profiles were acceptable for forensic purposes. In relation to the decomposition stage, the analyzed remains were grouped into four distinct stages: (1) fresh; (2) bloat; (3) decayed skeleton; or, (4) mummified.

The goal of the study was to observe that the treatment of the Sud Italian cemetery remains may promote the preservation of human bones and the success of the DNA analysis. The persistence of good bone condition in the burial environment is unusual as is the persistence of biomolecules in bone, but mummification is a common phenomena in South Italy cemeteries, where remains are buried in wooden coffins only for a limited time. After the disinterment, remains are co-located in stone niches. This procedure tends to protect bone as it appears much less deteriorated with respect to that collected from graves in which bodies are in contact with the ground.

Forensic Science, Human Identification, DNA Testing

B67 Development of a Portable Detection and Image-Processing System for Latent Fingerprints Using Time-Resolved Spectroscopy

Hidetoshi Kakuda, National Research Institute of Police Science, Physics Section, 6-3-1 Kashiwanoha, Kashiwa, Chiba 277-0882, JAPAN; Norimitsu Akiba, PhD, National Research Institute of Police Science, 6-3-1 Kashiwanoha, Kashiwa, Chiba 277-0882, JAPAN; Daisuke Imoto, MS, National Res Institute of Police Science, 6-3-1 Kashiwanoha, Kashiwa , JAPAN; Ken'ichi Tsuchiya, PhD, 6-3-1 Kashiwanoha, Kashiwa, Chiba 277-0882, JAPAN; Kenji Kurosawa, 6-3-1 Kashiwanoha, Kashiwa, Chiba 277-0882, JAPAN; Kenro Kuroki, PhD, 6-3-1 Kashiwanoha, Kashiwa, JAPAN; Shigeki Takeuchi, PhD, 2-1-1, Yabutaminami, Gifu-city 500-8974, JAPAN; and Osamu Shimoda, BS, 5-4-1, Yamatedouri, Tyuuou-ku, Hyogo Prefecture Police Headqrtrs, Kobe-city 650-8510, JAPAN*

After attending this presentation, attendees will understand how a portable detection and image-processing system for latent fingerprints using time-resolved spectroscopy is developed and applied to a preprocessed fingerprint.

This presentation will impact the forensic science community by presenting a report of a first attempt to develop a portable detection and image-processing system for latent fingerprints using time-resolved spectroscopy. It is expected that this system can be used for on-site detection and investigation of latent fingerprints.

Detection of fluorescence from latent fingerprints using time-resolved spectroscopy is one of the effective methods for visualizing latent fingerprints.[1,2] When a latent fingerprint is excited by irradiating with light, it emits fluorescence with a certain lifetime. This fluorescence provides information on the shape and components of the latent fingerprint. Since the light interacts with substances in a non-contact manner, an excitation of latent fingerprints by a light with modest power neither contaminates nor destroys the material evidence.

Although not only latent fingerprints but also background substances will emit fluorescence, it is possible to detect the fluorescence from latent fingerprints exclusively by using time-resolved spectroscopy. Time-resolved spectroscopy enables a detection of the light of interest by making use of differences in fluorescence lifetimes, which depend on substances. This spectroscopy is conducted by using a pulsed light source and a Charge-Coupled Device (CCD) camera with accurate temporal controls. After the image of the latent fingerprint is obtained, in some circumstances, it will be helpful to process it by a computer. Practically, since these devices include an optical system and tend to be large in size, the application of time-resolved spectroscopy to latent fingerprints has been limited to laboratory use.

This presentation reports on the development of a portable detection and image processing system for latent fingerprints using time-resolved spectroscopy in order to conduct on-site detection and investigation of latent fingerprints. It is noted that applying this system requires a preprocessing of a latent fingerprint for a fluorescence lifetime of several hundreds of µsec because the accuracy of temporal control of the programmable Matrox Iris GT, GT300C* CCD camera used in this system is limited to an order of µsec. This system adopts a Laser Diode (LD) wavelength 375nm as a light source and the programmable CCD camera equipped with a Central Processing Unit (CPU) for on-site data processing, such as image accumulations, to enhance Signal-to-Noise (S/N) ratio. Three timings concerning the LD and the CCD camera have to be provided: (1) light-emitting time of the LD; (2) delay time for opening a shutter of the CCD camera after the LD begins to emit; and, (3) exposure time of the CCD camera. Times (1) and (2) were set by a Sapphire Plus Pulse Generator, MODEL 9214+ delay pulse generator and time (3) was controlled by a program in the CCD camera. Both the pulse delay generator and the CCD camera were controlled and/or programmed by a tablet personal computer, which is connected to a portable monitor to enable on-site settings for instrumental conditions and checks of image processing results. One can also use a head-mount display instead of the monitor. These components are electrically supplied by lithium batteries. Constituents of the system weigh nearly 3kg in total and are portable.

This portable system was applied to a fingerprint preprocessed by Tris-Thienyl-Europium Chelate (T.TEC). The fluorescence lifetime of the T.TEC is approximately 500µsec. The light-emitting time of the LD was set to 400µsec and the exposure time of the CCD camera was adjusted to 200µsec. For the delay time for the CCD camera of 550µsec (i.e., the shutter of the CCD camera opens 150µsec after the LD emission ends), a fluorescence image of the latent fingerprint without the fluorescence from backgrounds was successfully obtained and clarified after 1,000 image accumulations and a histogram adjustment.

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Portability, Latent Fingerprint, Time-Resolved Spectroscopy

B68 Fingerprint Ridge Drift: An Undescribed Phenomenon

Josep De Alcaraz-Fossoul, PhD, University of Barcelona, Carrer Casanova, 143, 3rd Fl, North Wing, Faculty of Medicine - Forensic Genetics Laboratory, Barcelona, Catalonia 08036, SPAIN; Carme Barrot, PhD, University of Barcelona, Carrer Casanova, 143, 3rd Fl, North Wing, Faculty of Medicine - Forensic Genetics Laboratory, Barcelona 08036, SPAIN; Luke McGarr, BSc, The Corner House Business Centre, 2, Albert Road, Ripley DE53FZ, UNITED KINGDOM; Karen Stow, MSc, The Corner House Business Centre, 2, Albert Road, Ripley DE53FZ, UNITED KINGDOM; Katherine A. Roberts, PhD, CSU - Los Angeles, School of CJ & Criminalistics, Hertzberg-Davis For Sci Center, Los Angeles, CA 90032; Gregory G. Hogrebe, BS, 1581 Sherwood Village Circle, Placentia, CA 92870; and Manel Gené, PhD, University of Barcelona, Carrer Casanova, 143, 3rd Fl, North Wing, Faculty of Medicine - Forensic Genetics Laboratory, Barcelona 08036, SPAIN*

After attending this presentation, attendees will be informed about a newly discovered phenomenon named ridge drifting. Attendees will learn how to identify this feature that has never before been described.

This presentation will impact the forensic science community by introducing a new feature that describes changes in the topography of fingerprints that has previously gone unnoticed.

Fingerprint distortions have been described in the literature as being the result of applying a non-uniform pressure during deposition, combined with the inherent elasticity of friction ridge skin and the curved anatomy of the finger. In addition, fingerprint image deformations can also occur as a consequence of camera lens defects or are caused by the visualization/development process. These alterations of the fingerprint topography and of the acquired image are usually considered during a comparison process between a latent and an exemplar (inked or scanned) fingerprint.

The aforementioned distortions are characterized by: (1) being caused by an extrinsic force or action; (2) affecting entire areas of the deposition (or its image); and, (3) altering the overall flow of a series of contiguous ridges. In consequence, the ridges are "artificially" deformed, modifying the minutiae/ridge pattern and hindering comparisons. Unlike these identifiable types of deformations, a visual phenomenon that modifies the fingerprint at a ridge scale is described here and named "fingerprint ridge drift."

In an experiment to determine the degradation patterns of latent fingerprints, a sequence of impressions (eccrine and sebaceous) were deposited on non-porous surfaces (plastic and glass) by applying similar pressures for the same lengths of time. These were aged under three different light conditions (direct sunlight, shade, and darkness) for a period of six months, visualized with titanium dioxide over different time periods, and photographed. No further treatment or manipulation was performed on the samples. Photographs of fresh fingerprints were then compared with all of the aged prints. The analyses revealed that under certain environmental conditions, an individual ridge could randomly change its original position regardless of its unaltered adjacent ridges. This modification produced a change in the distribution of minutiae in the fingerprint at that specific location.

Currently, the causes of this phenomenon are not well understood. It is hypothesized that it could be the result of either: (1) a microscopic movement of the ridge over the non-porous surface by a diffusion (sliding) effect; or, (2) a process of degradation that affects only specific locations along the same ridge (selective degradation). In both cases, it would be a process exclusively associated with the intrinsic natural aging process of a latent fingerprint. At this time, the second option seems the most plausible. The study of superimposed fingerprint images has shown an apparent unchanged location of the affected ridges but a modification in their appearance.

It has been demonstrated that: (1) fingerprints from the same person can be slightly different if the factor "time" is included; (2) ridge drift occurs at random and has a very localized affect; (3) it is an intrinsic phenomenon not caused by external actions; and, (4) there is no obvious correlation between environmental conditions and ridge drift. To be confirmed, this discovery will help explain the detection of certain dissimilarities at the minutiae/ridge level between an aged latent fingerprint and its exemplar counterpart. Collaterally, the identification of this phenomenon will help determine more accurate "hits," identify potentially erroneous corresponding points, and rethink identification protocols, especially the common criteria of "no single minutiae discrepancy" for a positive match.

Fingerprint, Ridge, Aging

B69 Fingerprint Detection by Two-Photon Excitation With a Femtosecond Fiber Laser

Norimitsu Akiba, PhD, National Research Institute of Police Science, 6-3-1 Kashiwanoha, Kashiwa, Chiba 277-0882, JAPAN; Ryoya Takahashi, MS, JFE Techno-Res Corp, 1-Banchi, Kawasaki-cho, Chuo-ku, Chiba, JAPAN; Fumihiko Ichikawa, 1-banchi, Kawasaki-cho, chuo-ku, Chiba 260-0835, JAPAN; Akira Torao, 1-bncho, Kawasaki-cho, Chuo-ku, Chiba 260-0835, JAPAN; Naohiro Ishizawa, BS, Waseda University, 3-4-1 Okubo, Shinjuku-ku, JAPAN; Atsushi Nakamura, 3-4-1, Okubo, Shinjuku-ku, Tokyo, JAPAN; Takayuki Sota, 3-4-1, Okubo, Shinjuku-ku, Tokyo 169-8555, JAPAN; Hidetoshi Kakuda, National Research Institute of Police Science, Physics Section, 6-3-1 Kashiwanoha, Kashiwa, Chiba 277-0882, JAPAN; Daisuke Imoto, MS, National Res Institute of Police Science, 6-3-1 Kashiwanoha, Kashiwa, JAPAN; Ken'ichi Tsuchiya, PhD, 6-3-1 Kashiwanoha, Kashiwa, Chiba 2770882, JAPAN; Kenji Kurosawa, 6-3-1 Kashiwanoha, Kashiwa, Chiba 277-0882, JAPAN; and Kenro Kuroki, PhD, 6-3-1 Kashiwanoha, Kashiwa, JAPAN*

After attending this presentation, attendees will better understand a method of detecting fingerprints by two-photon excitation with an infrared femtosecond laser.

This presentation will impact the forensic science community by providing a non-contact, non-destructive, and non-invasive method to detect fingerprints. The proposed method does not affect DNA testing, which is one of the most important evidences in crime investigation.

Various methods such as using powder or chemical solutions are used for fingerprint detection. Conversely, methods using light such as laser and Light-Emitting Diode (LED) attract attention as a non-invasive method.[1] Fingerprint detection using fluorescence is an especially useful technique in forensic sciences. The visualization of latent fingerprints with an Ultraviolet (UV) -pulsed laser by time-resolved spectroscopy has been studied.[2] Even if the fluorescence of a background is strong, it is possible to visualize fingerprints by this time-resolved method using the difference of fluorescence lifetimes; however, it is a concern that fingerprints and deposits around them are damaged by UV light, which may affect other forensic examinations. It is important to use a less destructive method for criminal identification.

Therefore, this study focused on two-photon excitation with an infrared femtosecond laser. The visualization of latent fingerprints by two-photon excitation with a femtosecond solid-state laser is presented.[3] A compact system for on-site use is currently being constructed by replacing a large Ti:Sapphire solid-state laser to a small fiber laser, because mobility is required. In this study, fingerprint detection by two-photon excitation with a femtosecond fiber laser was performed in order to miniaturize the system.

Two-photon excitation was carried out with a femtosecond fiber laser and a fast-gated image Intensified Charge-Coupled Device (ICCD) camera. Repetition rates of the laser and ICCD camera were 50MHz and 500kHz, respectively. Pulses were picked with a frequency divider for the time-resolved spectroscopy. Damage to samples was reduced by scanning laser light with a galvano mirror and a resonant scanner. The objective lens was 20X (*N.A.*=0.4). Samples were placed on an X-Y-Z axis motorized stage. The excitation wavelength was 780nm and laser power was approximately 200mW to 300mW. Two-photon excitation at 780nm corresponds to one-photon excitation at 390nm. The irradiated area was approximately 50µm x 50µm.

At first, tests were performed using a green fluorescent pen. Gate width and exposure time were 10ns and 10s, respectively. A fluorescence spectrum whose peak of approximately 500nm was acquired. Since the peak of the emission spectrum was shorter than 780nm of excitation, it was confirmed that two-photon excitation occurred using this system with a fiber laser. Subsequently, the fluorescence spectrum of a fingerprint was measured. Gate width and exposure time were 2ns and 60s, respectively. A broad spectrum ranging from 400nm to 500nm was obtained, whose peak was approximately 450nm. It consists of the previous experiments using a femtosecond solid-state laser. Therefore, these results suggest that it was due to two-photon excitation of the fingerprint with the fiber laser. Furthermore, the lifetime of the inherent fingerprint was measured and found to be approximately 1.5ns. Finally, latent fingerprints on ceramics were visualized. Gate width and exposure time were 8ns and 20s, respectively. The ridge of a fingerprint was detected by two-photon excitation. In addition, fluorescence of a piece of skin was strongly detected. For future consideration, the researchers plan to visualize the fluorescence of additional fingerprints.

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Fingerprint, Fluorescence, Two-Photon Excitation

B70 Method Validation Parameters for Drugs and Explosives in Ion Mobility Spectrometry (IMS)

A. Bakarr Kanu, PhD, Winston-Salem University, Rm 311, Dept of Chem, WB Atkinson, 601 S Martin Luther King Jr Way, Winston-Salem, NC 27110*

After attending this presentation, attendees will develop an understanding of practical analytical figures of merit that could be used to distinguish a false positive response from an actual drug of abuse or explosive using IMS. A false positive response is when an interfering compound produces a response identical to that of a target compound.

This presentation will impact the forensic science community by providing data that will be very useful in solving problems with false positive response when IMS is used in the field. This presentation will add to research already performed in forensic instrumental analysis by broadening understanding of analytical figures of merit that could help unravel how well a target compound can be discriminated or separated from a false positive response.

A major problem with field measurements is the possibility of false positives. False positive responses result in loss of time, and further testing may be required to insure that target explosives or drugs of forensic interest may be absent or present in a given circumstance.[1] In the complex forensic world, it is becoming increasingly important to develop accurate parameters to reliably identify forensic samples. Method validation is the process of proving that an analytical method is acceptable for its intended purpose. Developing method validation parameters for a specific forensic compound may be the answer to developing an accurate identification marker for substances like drugs and explosives of forensic interest.

Certified reference materials of drugs and explosives samples were prepared at different concentrations and analyzed with an Electrospray Ionization-High-Performance Ion Mobility Spectrometry (ESI-HPIMS) to determine method validation parameters for drugs and explosives. The preliminary results have determined the following method validation parameters for drugs of abuse and explosives: conditional reduced mobility, limit of detection, limit of quantification, control chart, linearity, sensitivity, accuracy precision, range, resolving power, and reporting limit.[2-6]

In conclusion, the preliminary data suggests that method validation parameters, when correctly implemented, could be used as a unique identifier for forensic samples. The result holds great promise for detecting and identifying forensic samples and reducing false positive rates.

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Method Validation, Identity Confirmation, ESI-HPIMS

B71 Quantitation of Major Cannabinoids Found in Seized Marijuana Using Automated Headspace/ Solid-Phase Microextraction Coupled With Gas Chromatography/Mass Spectrometry (HS/ SPME-GC/MS)

Anastasia M. Brown, BS, 3207 Peddicoat Court, Woodstock, MD 21163; James D. Sweet, PhD, U.S. Customs and Border Protection, LSSD, 4150 Interwood S Parkway, Houston, TX 77032; and Jorn Chi-Chung Yu, PhD, Sam Houston State University, Dept of Forensic Science, Box 2525, Huntsville, TX 77341*

After attending this presentation, attendees will better understand using automated HS/SPME for the quantitation of Δ9-trahydrocannabinol, cannabidiol, and cannabinol from marijuana plant material.

This presentation will impact the forensic science community by providing an automated method for the quantitation of major cannabinoids through the direct headspace sampling of suspected marijuana samples. This presentation will enhance the applicability of HS/SPME-GC/MS to controlled substance analysis.

The term marijuana refers to the plant material of *Cannabis sativa* L. There are more than 60 natural cannabinoids found in marijuana. The primary psychoactive component is Δ9-Tetrahydrocannabinol (Δ9-THC). Other important components for forensic purposes in states with legalized marijuana include Cannabinol (CBN) and Cannabidiol (CBD). Current analytical methods for the detection of cannabinoids include solvent extractions followed by gas or liquid chromatography. Such methods have several limitations, including the use of hazardous solvents, the expense of said solvents, disposal of the waste generated from solvent use, and the time needed to perform such extractions. A solution that may eliminate such limitations is the use of an HS/SPME-GC/MS method to detect the cannabinoids found in marijuana samples.

In this research, an optimal automated HS/SPME-GC/MS method has been developed using cannabinoid standard reference materials and actual marijuana material samples. An internal standard of deuterated Δ9-THC (D3-Δ9-THC) and any standard reference samples used were placed in a vial and the solvent evaporated under a gentle air stream before analysis. The plant material was ground and sieved before being weighed out into sample vials. Unlike previous methods that would require the sample to be extracted with solvents before analysis, the HS/SPME-GC/MS method required the sample to be sealed in the sample vial and placed on a GC/MS autosampler that would carry out the HS/SPME extraction using a 23 gauge 100µm Poly(DiMethyl)Siloxane (PDMS) fiber and inject the extracted cannabinoids into the GC/MS. The optimized extraction temperature for cannabinoids was found to be 150°C for quantitative analysis, and the optimal extraction temperature was found to be five minutes. Regeneration of the PDMS fiber was achieved by heating the fiber to 250°C in the autosampler conditioning chamber during the run after the fiber was exposed to the inlet of the GC/MS.

Results from the optimized HS/SPME-GC/MS method show that quantitation of Δ9-THC, CBN, and CBD could be completed with an r^2 of 0.99 and combined accuracy of more than 95% when using an internal standard of D3-Δ9-THC. The quantitative ions used for Δ9-THC, CBN, and CBD were 310m/z, 295m/z, and 238m/z; 314m/z, 299m/z, and 231m/z; and 231m/z, 174m/z, and 121m/z, respectively. The same major cannabinoids can be seen with both traditional liquid extraction and HS/SPME methods; however, in some cases the HS/SPME-GC/MS method shows more cannabinoids than the liquid extraction. With a better sensitivity, faster sampling preparation, smaller sample quantity required, and cheaper supplies cost, HS/SPME-GC/MS quantitation can provide major advantages over traditional liquid extraction. Future research will include statistical analysis of the data collected from marijuana plant samples using an already optimized qualitative method and this quantitative method with the goal of being able to differentiate marijuana from different growers.

Marijuana, Quantitation, Solid-Phase Microextraction

B72 Analysis of Seized Hypodermic Syringes for Drug Content

Thomas A. Brettell, PhD, Cedar Crest College, 100 College Drive, Allentown, PA 18104; Robyn Pyle, MS, 777 W Germantown Pike, Apt 732, Plymouth Meeting, PA 19462; and Linda Burdick, BS, Bucks County Crime Laboratory, 850 Eagle Boulevard, Warminster, PA 18974*

After attending this presentation, attendees will have a better understanding of the results of analysis for controlled substances of 469 syringes seized by law enforcement and submitted to the Bucks County Crime Laboratory.

This presentation will impact the forensic science community by providing the results of one laboratory's experience of analyzing syringe residues for controlled substances by Gas Chromatography/Mass Spectrometry (GC/MS).

Drug paraphernalia is commonly submitted to forensic science laboratories for analysis of controlled substances. Paraphernalia seized by law enforcement often includes used syringes from drug abusers. The syringes are usually submitted to the laboratory with the needle intact. The proper submission of syringes should include a protective container labeled as a biohazard to protect anyone who must handle the evidence. Laboratories should have procedures in place to safely handle this type of evidence. It is a known fact that the sharing of syringes by intravenous drug abusers is a primary cause of the transmission of HIV and hepatitis B and C. Anyone who handles this type of evidence, particularly forensic chemists and law enforcement, is at risk of acquiring these diseases. For this reason, many laboratories choose not to analyze this type of evidence unless absolutely necessary.

However, there are a number of important reasons to analyze syringes submitted to crime laboratories. Laboratories may chose to have syringes analyzed because: (1) it is the only item in the case; (2) it may be the probable cause for arrest; (3) it may be essential for determining the cause of death in a death investigation; (4) possession of the contents of the syringe may be a significantly more serious offense than possession of other items in the case (e.g., a syringe with heroin vs. a bag of marijuana); or, (5) for other reasons specific to the case.

This presentation will report the results of analysis for controlled substances of 469 syringes seized by law enforcement and submitted to the Bucks County Crime Laboratory during the period of January 2014 through July 2015. The syringes were extracted with methanol. The methanol extract was evaporated, then reconstituted with five drops of methanol and divided into two vials, which were analyzed by GC/MS on separate instruments with different conditions. One-microliter injections of the methanol extracts were analyzed by GC/MS on an Agilent® 7890B gas chromatograph equipped with a 7693 autosampler interfaced to a 5977A mass selective detector. The column used was a 30m x 0.25mm x 0.25µm HP-5MS. The inlet temperature was 290ºC, the transfer line temperature was 250ºC, and the source temperature was 230ºC. One-microliter injections of the second aliquot were analyzed on a Perkin® Elmer® gas chromatograph model Clarus® 500 interfaced to a Clarus® 500S Perkin® Elmer® mass spectrometer. The column used was a 15m x 0.25mm x 0.25µm ELITE-5MS. Helium was used as the carrier gas for both columns with a flow of 1.0mL/min and linear gas velocity set at 38cm/sec. The inlet temperature was 290ºC, the transfer line temperature was 270ºC, and the source temperature was 250ºC. The mass scan range was set at 40m/z-500m/z for all samples. The column temperature program was optimized on both columns to ensure baseline resolution of heroin and 6-monoacetylmorphine (6-MAM). The initial column temperature was 130ºC with a hold time of 2.0 minutes, then the oven temperature was increased at 15ºC/min to 250ºC without a hold time and increased again at 15ºC/min to 320ºC with a final hold time of 3.0 minutes. The samples were run in split mode and with a 50:1 split ratio.

Of the 469 syringes analyzed, 71% were positive and 62% of the 294 cases analyzed were positive. The most common drug identified was heroin (46%). Other drugs identified were morphine (11%), cocaine (5%), methamphetamine (3%), fentanyl (1%), oxycodone (1%), and buprenorphine (1%) as well as various cutting agents, heroin metabolites and naturally occurring opiate alkaloids. Caffeine (16%) was the most common non-controlled substance detected.

Controlled Substances, Syringes, GC/MS

B73 The Development of a Novel Color Test for Improved Detection of Synthetic Cathinones

Charles R. Cornett, PhD, University of WI Platteville, Dept of Chemistry, Platteville, WI 53818; Nicole Kloepfer, University of Wisconsin-Platteville, 1 University Plaza, Platteville, WI 53818; Brooke Tashner, BS, University of Wisconsin-Platteville, Dept of Chemistry, 1 University Plaza, Platteville, WI 53818; and Tsunghsueh Wu, PhD, University of Wisconsin-Platteville, Dept of Chemistry, 1 University Plaza, Platteville, WI 53818*

After attending this presentation, attendees will better understand the development of a new color test for synthetic cathinones (aka "bath salts") and the scope of the test with respect to future derivatizations.

This presentation will impact the forensic science community by introducing a more reliable, presumptive means of detecting synthetic cathinones in the field and laboratory.

Color tests have been used by criminalists for decades to provide a rapid, inexpensive means of determining if an unknown compound merits further investigation. Law enforcement officers rely upon commercially produced presumptive color tests to determine probable cause for arrest and subsequent substance identification by crime laboratory personnel and techniques. Following a positive field presumptive test, the evidence (a potential controlled substance) is sent to the crime laboratory for further processing. This involves an indicative test and finally a confirmatory test. Color tests may also be used by crime laboratory personnel as a presumptive test.

Designer and emergent illicit drugs have entered the market at a rapid pace in the past five years, and synthetic cathinones (bath salts) are one example of these drugs. Synthetic cathinones are beta-keto phenethylamine derivatives that produce pharmacological effects similar to the Schedule I substances such as cathinone, methcathinone, and 3,4- Methylenedioxymethamphetamine (MDMA). The parent cathinone structure is easily derivatized at any of four sites to generate analogues not yet regulated by state legislation or the **Drug Enforcement Administration** (DEA) Scheduled substance list. Unfortunately, the derivatization of the cathinone structure has resulted in presumptive color tests that are often not detecting new bath salts, are providing different colors for different cathinone derivatives, or require several vials of various component reagents to be effective. It stands to reason that a reliable color test for synthetic cathinones capable of operating with great sensitivity over a wide range of environmental conditions and packaged in two or fewer ampules would constitute a significant advancement in the field.

This research and development has produced a two-stage color test utilizing proprietary reagents WuCo 1 and WuCo 2 in sequence. These reagents utilize organic dyes containing groups such as a sulfonic acid along with organic solvents and buffers described in this presentation. Experiments conducted demonstrate that the reagents are well suited for deployment and use in the field by law enforcement as well as the bench top as a presumptive test. The two-stage color test provides a consistent yellow color in the organic layer, and there are no impediments to its use in the field in a two-ampule system. In addition, the 20 synthetic cathinones tested can be detected in amounts ranging from microgram quantities to more than 30 milligram per testing package. Furthermore, the reagents produce effective results in a wide variety of environmental conditions, including at temperatures from -3°C to 40°C. This presentation details the 100% effectiveness in presumptive indication of a variety of synthetic cathinones.

Bath Salts, Synthetic Cathinone, Color Test

B74 Further Characterization of Opiates in Poppy Pod Tea Preparations

Angela S. Mohrhaus, BS, 6751 Steger Drive, Cincinnati, OH 45237; Heather A. McCauley, BS, 6751 Steger Drive, Cincinnati, OH 45237; Jill M. Robinson, MFS, 6751 Steger Drive, Cincinnati, OH 45237; and Samuel R. Gratz, PhD, 6751 Steger Drive, Cincinnati, OH 45237*

WITHDRAWN

B75 Rapid Screening of Seized Drugs Using Direct Analysis in Real-Time Mass Spectrometry (DART®-MS)

Yuriy Uvaydov, MS, 99 Tenth Avenue, Ste 721, New York, NY 10011*

After attending this presentation, attendees will understand how DART®-MS can be applied for the screening and identification of commonly encountered drugs of abuse. Specifically, this presentation will demonstrate the advantages and limitations of DART®-MS over traditional instrumental techniques such as Gas Chromatography/Flame Ionization Detector (GC/FID), Gas Chromatography/Mass Spectrometry (GC/MS), and Liquid Chromatography/Mass Spectrometry (LC/MS).

This presentation will impact the forensic science community by explaining how DART®-MS can be effectively applied as a screening and identification technique for the presence of illicit drugs in single- and/or multi-unit submissions of evidence.

There are currently several existing instrumental techniques that can be applied for screening and identification of commonly encountered drugs of abuse. Typically, analysis of seized drug evidence consists of a series of presumptive and confirmatory tests. Traditional presumptive tests may include color, microcrystal, Thin-Layer Chromatography (TLC), GC, and/or Ultra High-Performance Liquid Chromatography (UHPLC). Confirmatory techniques, on the other hand, may include GC/MS, LC/MS, Fourier Transform Infrared/Attenuated Total Reflectance (FTIR/ATR), and/or Nuclear Magnetic Resonance (NMR). The continuing demand for faster, more efficient methodologies demonstrates the need to incorporate newer analytical instrumentation into the classical workflow.

DART®-MS is a relatively new ionization technique for mass spectral analysis of compounds. DART®-MS allows for ambient ionization of small molecules from different samples without sample preparation. The samples are directly introduced into the ion source using tweezers or capillary glass tubes and are desorbed from sample surface by flow of heated nitrogen or helium while being ionized. In conjunction with the High Resolution Accurate Mass Spectrometer (HRAMS), DART®-MS can deliver rapid results with accurate mass determinations and highly specific mass-to-charge spectral data.

The purpose of this work was to develop a method for rapid detection of drugs (target or unknown) utilizing HRAM coupled to a DART® ionization source. In this study, a 24-second screening method was developed for the detection of commonly encountered illicit drugs. Mass spectral acquisition of data was performed in positive mode utilizing a DART®-Simplified Voltage and Pressure (SVP) ion source with Thermo Fisher's Exactive Plus Mass Spectrometer. Powdered samples were introduced directly into the ion source via glass capillary tubes utilizing the Dip-It linear rail system that is connected to the DART®-SVP ion source. Data acquisition included fragmentation patterns utilizing Source-Induced Dissociation (SID), generated at 30eV, 60eV, and 100eV.

Preliminary results demonstrated successful identification of controlled substances in various drug mixtures. For example, unknown samples containing heroin, fentanyl, and dipyrone were correctly identified based on the characteristic peaks with the assigned chemical formulas. Similarly, unknown samples containing cocaine, levamisole, and phenacetin were also correctly identified. The results also showed that DART®-MS identifications of major sample components in other various drug mixtures were correctly identified. The identification of minor components presented a challenge and may require further optimization of the HRAM/DART®-MS acquisition parameters. Overall, this approach is expected to decrease analysis time, increase efficiency in screening multi-unit drug submissions of evidence, while maintaining cost-effectiveness and achieving valid reproducible data.

DART®-MS, Forensic Drugs, Rapid Screening

B76 Evaluation of a Direct Analysis Portable Mass Spectrometer (MS) for the Detection of Drugs and Related Substances

Nichole D. Bynum, MS, RTI International, 3040 Cornwallis Road, Bldg 3, Research Triangle Park, NC 27709; Katherine N. Moore, MS, 2526 Folsom Lane, Morrisville, NC 27560; Zachary E. Lawton, BS, Illinois State University, Campus Box 4160, Normal, IL 61790-4160; Christopher C. Mulligan, PhD, Illinois State University, Dept of Chemistry, Campus Box 4160, Normal, IL 61790-4160; Megan Grabenauer, PhD, RTI International, 3040 E Cornwallis Road, PO Box 12194, Research Triangle Park, NC 27709-2194; and Jeri D. Ropero-Miller, PhD, RTI International, 3040 Cornwallis Road, PO Box 12194, Bldg 7, Rm 211, Research Triangle Park, NC 27709*

WITHDRAWN

B77 Analysis of Cannabis for the Presence of Pesticides and Adulterants With High Resolution Tandem Mass Spectrometry

Werner Bernhard, DSc, IRM University of Bern, Buehlstrasse 20, Bern CH-3012, SWITZERLAND; Stefan Koenig, PhD, Institute of Forensic Medicine, Buehlstrasse 20, Bern 3012, SWITZERLAND; Susanne Nussbaumer, PhD, Institute of Forensic Medicine, Buehlstrasse 20, Bern 3012, SWITZERLAND; and Wolfgang Weinmann, PhD, Institute of Forensic Medicine, Bühlstrasse 20, Bern 3012, SWITZERLAND*

After attending this presentation, attendees will be aware that pesticides are found in seized cannabis. These compounds are used in order to promote growth and prevent insects/fungi from destroying the plants or reducing the yield. Acquisition of mass spectra in Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra (SWATH) mode on a quadrupole Time-Of-Flight (qTOF) instrument allows for unambiguous determination of the pesticides present in marijuana samples.

This presentation will impact the forensic science community by presenting a rapid and reliable analytical method to detect pesticides in marijuana that were used during the cultivation and were not degraded before harvesting.

Goals: Cannabis consumption is observed as a wide-spread phenomenon and in some cases the cannabis users also consume toxic contaminants without being aware of possible side effects and long-term adverse health effects. In order to assess the exposure of cannabis users to pesticides and adulterants (e.g., synthetic cannabinoids), a specific High-Performance Liquid Chromatography (HPLC) qTOF method was developed for herbal marijuana samples.

Methods: The sample collection was performed during the routine forensic analysis of seized drug materials. The plant samples were then processed via the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method and further cleaned by dispersive solid phase extraction. The prepared samples were injected onto a core shell column (Biphenyl, 50mm x 2.1mm, 2.6µm), analyzed on a qTOF instrument, and measured by non-targeted acquisition (SWATH). Typical run times of 20 minutes from injection to injection were achieved.

Results: A total of 151 marijuana samples from different locations within Switzerland were analyzed by this newly developed method. These samples were collected during the past 12 months and stored at room temperature in the laboratory. The scan parameters were optimized in order to meet the complexity of these plant samples. The following parameters were found to be most favorable for significant library hits: scan range from 50Da to 950Da, scan windows of 25Da, scan time of 35msec for each scan window,and collision energy of 35eV±15eV (collision energy spread). For unambiguous identification accurate mass of the precursor ions, Tandem Mass Spectrometry (MS/MS) spectra and retention time was used as criteria. These criteria were previously determined by injection of clean standard solutions of the pesticides into this system.

In 12 of these 151 samples, pesticides were found in various concentrations. Among these pesticides the following compounds were identified: propamocarb, imidacloprid, dimethoate, Diethyltoluamid (DEET), and metalaxyl.

Conclusions: A straightforward and reliable method of analysis was developed for the detection of pesticides in marijuana as is already established as standard procedure for food and drinking water. These pesticides can represent additional health hazards for marijuana smokers and therefore a quality control for legal marijuana is certainly needed.

Pesticides, Marijuana, HR Tandem Mass Spectrometry

B78 Differentiation of Seized Marijuana Samples Using Automated Headspace/Solid-Phase Microextraction Coupled to Gas Chromatograph/Mass Spectrometer/Flame Ionization Detector (HS/SPME-GC/MS/FID) and Principal Component Analysis (PCA)

Jessica Winborn, BS, 2405B FM 1375, E, Huntsville, TX 77340; James D. Sweet, PhD, U.S. Customs and Border Protection, LSSD, 4150 Interwood S Parkway, Houston, TX 77032; and Jorn Chi-Chung Yu, PhD, Sam Houston State University, Dept of Forensic Science, Box 2525, Huntsville, TX 77341*

After attending this presentation, attendees will better understand automated HS/SPME-GC/MS/FID methodology and its application to the differentiation of different marijuana samples.

This presentation will impact the forensic science community by providing an automated method for the differentiation of different marijuana seizures. This presentation will enhance the applicability of HS/SPME-GC/MS/FID to controlled substance analysis and allow agencies to gather intelligence about drug distribution networks.

The term marijuana refers to the plant material (mostly leaves and buds) of *Cannabis sativa* L. There are more than 60 natural cannabinoids found in marijuana. The primary psychoactive component is Δ9-Tetrahydrocannabinol (Δ9-THC). Marijuana is federally a Schedule I controlled substance, but on a state level it ranges from fully illegal to fully legal. The change in legislation has raised new concerns for law enforcement. One of these concerns is whether legally grown marijuana is being diverted out of states where it is legal for recreational use to ones in which it is illegal. The ability to track the flow of marijuana to and within different jurisdictions will be an important tool for law enforcement officials.

In this study, an HS/SPME-GC/MS/FID method has been applied to seized marijuana samples to analyze their cannabinoid profile in order to assess common origin between seizures. The plant material was ground and sieved before being weighed out into sample vials. The vials were placed on a GC/MS autosampler that would carry out the HS/SPME extraction using a Polydimethylsiloxane (PDMS), 23 gauge, 100µm absorbent fiber and the extracted sample was injected into the GC/MS. The extraction temperature for cannabinoids was 150°C and the optimal extraction time was five minutes. Regeneration of the PDMS fiber was achieved by heating the fiber to 250°C in the autosampler conditioning chamber during the run after the fiber was exposed to the inlet of the GC/MS. The resulting cannabinoid profiles for each seizure of marijuana were analyzed using Principal Component Analysis (PCA) to assess the differences between them.

Results from this study show the analysis of cannabinoid profiles using HS/SPME-GC/MS and PCA to have great potential for being able to differentiate different marijuana samples. One of the seizures used in this study was differentiated from the other two. The remaining two seizures showed overlap between them. Future research will include the analysis of other non-cannabinoids present in the chemical profile of marijuana samples to improve the discriminatory power of this method.

Marijuana, HS/SPME, Principal Component Analysis

B79 The Effects of Ultraviolet (UV) Radiation on Time-Dependent Changes in the Composition of Latent Fingerprints

Allyson K Digmann, BS, Southeast Missouri State University, Dept of Chemistry, 1 University Plaza, Cape Girardeau, MO 63701; and James W. McGill, PhD, Southeast Missouri State University, Dept of Chemistry, One University Plaza, Cape Girardeau, MO 63701*

After attending this presentation, attendees will understand how UV radiation affects the perceived age of latent fingerprints, with age referring to the time since the fingerprint was left on a surface.

This presentation will impact the forensic science community by providing fingerprint analysts with additional information to use when working a case. Previous research has been performed to determine the time since deposition of a fingerprint as well as the age of the fingerprint donor, but there are many environmental factors that have yet to be considered, with UV radiation being one of those factors. This information can be used to help investigators include or exclude fingerprints from a crime scene.

Fingerprint ridge patterns have been demonstrated to be unique to an individual for many years. While latent fingerprints found at a crime scene are useful in including or excluding an individual from being present at a crime scene, they currently provide little information without an exemplar fingerprint for comparison. Studies have been undertaken to determine if more information can be established from a latent print, such as characteristics of the person who left the fingerprints, as well as how long the fingerprint has been on that surface, also known as time since deposition. Current research suggests that the time since deposition can be determined under controlled laboratory conditions and also that the donor age can be determined to within five years for suspects more than 20 years old; however, there are many environmental elements that must also be considered for real-world application.

In this study, there are two variables: time since deposition and UV radiation levels. Ongoing research suggests that time-dependent changes in the fingerprint composition can be used to estimate the time since deposition in ambient laboratory conditions. For this study, UV radiation wavelengths were controlled to determine if UV radiation affects the time-dependent changes of the fingerprint composition. There were four time-since-deposition groups: one day, one week, one month, and four months. The ages used ranged from 18 years old to 24 years old. For this study, donor age was not a variable in order to achieve more specific results for the time since deposition. For each donor, 24 fingerprints were taken and divided into two trials of 12. Those 12 prints were further divided into the four time-since-deposition groups, with each group having three prints, one with no added radiation, one with added UVA radiation, and one with added UVB radiation. All prints were on the same surface: aluminum. The added radiation levels were controlled using UV lamps. This allowed for each print to only be exposed to the added radiation or not to be exposed to any radiation at all.

To determine if the UV radiation affected the time since deposition, the radiated fingerprints were compared with the non-radiated prints for the corresponding time-since-deposition group. After reaching the target time since deposition, the prints were tested using Fourier Transform Infrared Spectroscopy (FTIS) and Raman spectroscopy to determine the chemical composition of the fingerprint over time, followed by chemometric analysis to identify time-dependent changes in each fingerprint. These changes were then compared to ascertain the correlation between UV radiation and any changes in the time since deposition in the prints to which UV radiation was added.

Fingerprint, Time Since Deposition, Ultraviolet Radiation

B80 Effects of Donor Age and Water Exposure on the Quality of Oil Red O-Stained, Water-Exposed Latent Prints

Kitrina D. Skaggs, BA, Southeast Missouri State University, Dept of Chemistry, 1 University Plaza, Cape Girardeau, MO 63701; James W. McGill, PhD, Southeast Missouri State University, Dept of Chemistry, 1 University Plaza, Cape Girardeau, MO 63701; and Madalyn R. Robinson, BA, Southeast Missouri State University, Dept of Chemistry, 1 University Plaza, Cape Girardeau, MO 63701*

After attending this presentation, attendees will understand how the age of the fingerprint donor affects the quality of Oil Red O-stained latent prints that have been previously wet with water. Attendees will also understand how the quality of Oil Red O-stained latent prints that have been previously wet with water is affected by exposure to three common aqueous mixtures.

This presentation will impact the forensic science community by expanding the understanding of the applicability, scope, and limitations of Oil Red O lipid staining reagent for visualizing latent fingerprints on paper that has previously been wet with water. A better understanding of these variables — including donor age and exposure to different aqueous mixtures — will guide the user in application of this reagent in casework and validation studies.

Oil Red O is a soluble lipid stain that was reported in 2004 to be efficacious for partitioning into and staining sebaceous materials in latent fingerprints. It is considered particularly useful for visualizing latent fingerprints on porous surfaces that have been wet with water, such as wet currency or paper. While other water-soluble components such as salts and amino acids may be washed away by exposure to water, water-insoluble fatty components of the fingerprint residues are much more resistant to removal by water and often persist after exposure to water.

In seeking to better understand the utility, scope, and limitations of Oil Red O stain reagent in visualization of lipidic components of latent prints, the present study examines the effects of two variables on the quality of latent prints stained using Oil Red O: Variable 1 — exposure to moving water of three varieties (tap water, Mississippi River water, and water containing laundry detergent); and, Variable 2 — the age of the latent print donor (from 1 to more than 60 years old). These two variables are expected to affect the lipid composition of latent fingerprints in a number of ways.

Water is known to have a much smaller effect on sebaceous materials deposited on a surface than on inorganic constituents such as salts; however, different types of water might be expected to affect the lipid content more than pure water. For example, tap water contains ions, disinfectants, and disinfectant by-products that might interact physically or chemically with sebaceous materials to alter their makeup. River water contains sediment that might act as a physical abrasive, thereby mechanically removing sebaceous materials from a surface. Finally, laundry detergent is used as a surfactant to aid in removal of greasy stains and soils from fabrics in the presence of water. It would reasonably be expected to remove sebum from paper as well.

Donor age is well-established as a variable affecting the chemical composition of latent fingerprints. In fact, fingerprint donors have been classified and donor age predicted based on analysis of the chemical composition of the print residues. Children are known to have more, shorter chain, free fatty acid components in their fingerprints, leading to higher volatility and thus shorter persistence in abduction cases. Teenagers and young adults typically have oilier skin, characterized by longer chain lipids, fatty, waxy, and cholesteryl esters, which decrease in concentration into young adulthood and middle age. Finally, older adults are characterized by decreasing amounts of sebum and drier skin. Since Oil Red O partitions into lipid material, these changes should manifest in varying quality of stained fingerprints.

Fingerprint samples were collected on pieces of various papers, including typical office laser printer paper and cotton blend papers. Subjects deposited fingerprints in triplicate on various papers, which were then treated by exposure to moving water solutions using a mechanical agitator. The latent prints were then visualized using a prescribed staining procedure with Oil Red O dye solution. The stained prints were photographed and their quality was assessed using two methods: qualitative stain color intensity and visibility of a set of minutiae predetermined from examination of inked prints of the donor subjects. Effects of varying these parameters will be reported.

Oil Red O, Fingerprint Quality, Donor Age

B81 Fingerprint Loss in a Cancer Patient With No Side Effects

Luciano Garofano, PhD, Accademia Italiana di Scienze Forensi, Via G. D'Annunzio n.9, Parma 43100, ITALY; Francesca Negri, MD, PhD, Oncology Unit, University Hospital of Parma, University Hospital, Parma 43100, ITALY; Annamaria De Giorgi, MD, Oncology Unit, University Hospital of Parma, University Hospital, Parma 43100, ITALY; and Luigi Bisogno, Via N. Capuano, 10, Castel San Giorgio (Sa) 84083, ITALY*

After attending this presentation, attendees will better understand the effects of chemotherapy on fingerprints.

This presentation will impact the forensic science community by raising awareness of the effects of chemotherapy, which may cause the loss of papillary fingerprints.

The case of a 75-year-old man with stage III colon cancer who received a six-month adjuvant chemotherapy treatment with capecitabine and oxaliplatin with no side effects is presented. During the first six-month follow-up visit, he was healthy, but as he was leaving, he incidentally asked if chemotherapy could have altered his fingerprints. In fact, his bank had recently denied access because his fingerprints were not recognizable. Moreover, he had to switch off his iPhone® Touch ID because it would not work anymore.

As it was known that he had not experienced any grade of skin toxicity during chemotherapy, he was asked to be fingerprinted for comparison, but the older fingerprints were unavailable, which represented a big issue. After two months of unsuccessful research and attempting to find a solution, the patient reported that he was again able to enter his bank. By comparing his fingerprints during and after chemotherapy, the loss of fingerprints was confirmed due to the presence of thinner ridges, a narrowing of the height between the top and bottom of the groove, and the absence of the relief, thus creating problems in scanner readings.

Cases of fingerprint loss during chemotherapy have previously been reported and have always been associated with some degree of hand/foot syndrome; however, this particular situation is the first-ever reported case with no skin toxicity.

Two practical conclusions can be drawn from this information: (1) fingerprint alteration might also arise in the absence of other clinical signs and this can be a handicap in patients taking chemotherapy drugs; therefore, they should be aware of this possibility; and, (2) this process is reversible and therefore needs to be brought to the attention of banks and the police force in order to avoid unpleasant consequences.

Fingerprint, Chemotherapy, Loss

B82 Pyrolysis Products of BK-2C-B and BK-2C-I, Beta-Keto Analogs of 2,5-Dimethoxy-4-Bromophenethylamine

Pierce V. Kavanagh, PhD, Trinity College Dublin, College Green, Pharmacology & Therapeutics, Trinity Centre, SJH, Dublin, IRELAND; Kelly B. Texter, BS, University of Alabama-Birmingham, 15950 Meadow King Way, Milton, GA 30004-8814; Rachel Waymack, College of William and Mary, Ewell Hall, Williamsburg, VA 23187-8795; and Elizabeth A. Gardner, PhD, UAB Department of Justice, UBOB 210, 1530 3rd Avenue, S, Birmingham, AL 35294-4562*

The goal of this presentation is to develop a better understanding of the products that result from the pyrolysis of the ß-keto analogs of the 2,5-dimethoxyphenethylamines: (1) 2-amino-1-(4-bromo-2, 5-dimethoxyphenyl)ethan-1-one (ßk-2C-B); and, (2) 2-amino-1-(4-iodo-2, 5-dimethoxyphenyl)ethan-1-one (ßk-2C-I).

This presentation will impact the forensic science community by identifying the products produced during pyrolysis of the beta-keto analogs of the 2,5-dimethoxyphenethylamines and their potential toxicity. Detecting these products in biological samples may also aid toxicologists in the identification of the drug and the route of administration.

The family of psychedelic phenethylamines called "2Cs" were first synthesized in the 1970s by Alexander Shulgin and published in his book, *Phenethylamines i Have Known and Loved* (PiHKAL).[1] The chemical structure of the 2C compounds has methoxy groups at the 2 and 5 positions of a phenethylamine ring. The acronym of 2C refers to the two carbon atoms between the benzene ring and the amino group.

Although 4-bromo-2,5-dimethoxyphenethylamine (2C-B) was scheduled in 1995, the beta-keto analog did not appear as a legal high until 2013.[2,3] The only structural difference between 2C-B and *bk*-2C-B is the addition of the beta keto group at the carbon beta to the amino group. Even though 2-amino-1-(4-bromo-2,5-dimethoxyphenethyl)ethanone (*bk*-2C-B) was first synthesized in 1974, there is little scientific data available on its use as a recreational drug. The first literature report was a 2004 study of β-oxygenated analogs of 1-(4-Bromo-2,5-dimethoxyphenyl)-2-aminopropane.[4] Power et al. recently reported on the synthesis and *in vitro* metabolism of *bk*-2C-B and compared it to a purchased sample.[5] They also determined that *bk*-2C-B reacts in the injector port of the Gas Chromatography (GC) to form decomposition products, namely 1-(4-bromo-2,5-dimethoxyphenyl)-ethanone, and a dimer.[2] In this work, *bk*-2C-B was pyrolyzed in order to identify the substances inhaled when the drug is smoked.

For the pyrolysis reaction, a sample of *bk*-2C-B (5mg-9mg) was loaded into an aluminum foil boat, placed in the bottom of a 20ml crimp vial, and heated with a disposable lighter for approximately 30s until yellow vapors formed in the vial. The foil cup was removed and the flask was rinsed with acetonitrile to dissolve the residues for GC/MS. The derivication was an acetylation performed using 100 µL of acetic anhydride and heating at 70°C for three hours. The vial was cooled to room temperature and the contents analyzed by Gas Chromatography/Mass Spectrometry (GC/MS). For Trimethylsilyl (TMS) derivatization, the residue was taken up in N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) /toluene (1/1) and analyzed by GC/MS.

Twelve products were detected and confirmed by comparison to standards synthesized at Trinity College Dublin. They are primarily the result of bond cleavage and halogenation. Unlike cocaine and methamphetamine, most of the *bk*-2C-B decomposed during pyrolysis. The primary products from the pyrolysis of *bk*-2C-B are 1-(4-bromo-2-hydroxy-5-methoxyphenyl)-ethanone and 1-(4-bromo-2,5-dimethoxyphenyl)-ethanone. The Iodo analog were also detected after the pyrolysis of *bk*-2C-I. Four additional analogues to the *bk*-2C-B pyrolysis products and one distinct structure, 1-(2,5-dimethoxyphenyl)ethan-1-one) have been identified for *bk*-2C-I.

The pyrolysis of *bk*-2C-B produces chemicals that have been tested as fungicides and proton chromophores. The 1-(2,5-dimethoxyphenyl)ethan-1-one) is a skin, eye, and respiratory irritant, an ingredient in pesticides, and has been tested as a potential anti-cancer agent. Analysis is ongoing.

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2,5-Dimethoxyphenethylamine, Pyrolysis, Legal High

B83 Assessing the Utility of Detrital Quartz Surface Textures and Feldspar Mineral Chemistry for Forensic and Intelligence Applications

Jack Hietpas, PhD, FBI-ORISE, 2501 Investigation Parkway, Quantico, VA 22135; JenaMarie Baldaino, BS, 5012 Coachmans Carriage Terrace, Glen Allen, VA 23059; JoAnn Buscaglia, PhD, FBI Laboratory, CFSRU, 2501 Investigation Parkway, Quantico, VA 22135; Garrett McMahon, BS, ORISE-FBI, 2501 Investigation Parkway, Quantico, VA 22135; and Libby A. Stern, PhD, FBI Laboratory - CFSRU, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, attendees will better understand the strengths and limitations of the light mineral fraction of soils as metrics for the characterization and discrimination of soil and dust samples.

This presentation will impact the forensic science community by demonstrating the techniques for the exploitation of quartz and feldspar as quantitative methods for the characterization of soil evidence, which may provide valuable prosecutorial evidence as well as lead identification for forensic investigations and intelligence purposes.

The mineral assemblages in soils are commonly utilized to make associations between people, objects, and locations. For forensic and provenance studies, the light mineral fraction (ρ<2.9g/cm3) of soils is not considered to be as discriminating as the heavy mineral fraction (ρ>2.9g/cm3); however, the light minerals typically account for 90%-99% of the fine-to-coarse (>50µm) mineral fraction and are, therefore, more likely to be present in sufficient quantity in the very small (<<1g) sample sizes commonly encountered in casework. The goal of this research was to investigate metrics to exploit potentially valuable information from the more abundant light mineral fraction of soils. To this end, 11 surface soil samples were collected from regions with distinct bedrock/surface geology, ecoregion, and physiography. This project investigated the two most common minerals in the light fraction of soils and sediments (quartz and feldspar). Interpretation of quartz surface textures using high-resolution Scanning Electron Microscopy (SEM) can be used to constrain environmental (littoral, subaqueous, eolian, among others) and petrogenetic (sedimentary, igneous, and metamorphic) origins.[1]

In addition, the major element composition of the detrital feldspar minerals was measured using quantitative SEM Silicon Drift Detector-Energy-Dispersive X-Ray Spectroscopy (SDD-EDS). The elemental composition of feldspar is highly variable and is closely related to the rock type from which it formed and thus may be used to infer the source rock type from which these minerals were derived.[2-5] Isolated grains were embedded in epoxy and subsequently ground and polished to expose the interior of the grains; quantitative analysis was performed using Desktop Spectrum Analyzer (DTSA-II), an open-source software package available from the National Institute of Standards and Technology (NIST).[6] Smithsonian Institution mineral and glass standards were used for calibration and as reference spectra for quantitative analysis.

Preliminary results show that the quartz surface textures (n=30-40 grains/sample) can be used to distinguish between several of the analyzed soil samples. In addition, the observed quartz surface textures correlate well with the geological and environmental settings from which the soils were collected. The elemental composition of isolated detrital feldspar grains (n=50-100 grains/sample) provided valuable information concerning the identity of the eroding local bedrock from which the soils were derived; however, several of the samples in this limited collection did not contain a sufficient number of feldspar grains for a meaningful, robust bedrock (source) assessment. The results from this research may provide new metrics and information for domestic criminal investigations and intelligence purposes.

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Forensic Geology, Light Minerals, Provenance

B84 Chemical Pattern Recognition: What Can Be Extracted From Geo-Located Spectroscopic Data Sets?

Sergey Mamedov, PhD, 3880 Park Avenue, Edison, NJ 08820*

After attending this presentation, attendees will understand the usage of X-Ray Fluorescence (XRF) and Raman microscopy in applications of sand analysis, an important element in forensic identification of these materials.

This presentation will impact the forensic science community by serving as a key aspect of sand/soil analysis and as an example of a practical application of XRF and Raman spectroscopy to materials identification.

XRF and Raman spectroscopies are useful tools for analyzing substances and confirming their identity with little or no sample preparation. XRF provides information about the elemental composition of the material whereas Raman spectroscopy gives molecular information. Both techniques enable the recording of not only spectra of small sand particles (as small as 50-100 microns) but also hyper-spectral imaging and collection of average spectra over a given area. Multivariate Analysis (MVA) can produce chemical distributions of elements and/or material classification based on Principal Component Analysis (PCA) and Partial Least Square Discriminative Analysis (PLSDA) in particular with association between elements that can aid in the identification of bonded phases. The analysis of micro-XRF data and Raman data of sand taken from different areas can be used to identify geographic locations.

XRF and Raman analytical microscopes were used in this study. XRF spectra of the materials were collected using a 50keV acceleration voltage with an X-ray spot size of 1.2mm. Two excitation wavelengths (532nm and 633nm) were used to collect Raman spectra with a spot size of 10-100 microns, depending on the material. Samples of sand (10g in weight) were ground and the resulting powder was used for XRF and Raman measurements.

The spectra of sand from different locations (the United States, Europe, and the Middle East) were collected and analyzed by micro-XRF and Raman spectroscopy. XRF analysis was performed in the range of 1.00keV-40.96keV (<400 spectra). Because only few spectra have additional features in the energy range above 15keV, spectra were truncated and analysis was performed in the spectral range of 1.00keV-15keV. A standard Fixed Point Multiplication (FPM) algorithm was used to calculate the concentration of oxides in all samples. This concentration set was used to build a data set for PCA and PLSDA. Correlation between classifications based on spectral analysis and concentration analysis will be shown. Raman spectra were collected in the range of 100cm-1-3500cm-1 and MVA was applied to these spectra to extract differences in connection with geolocation. The data show that MVA allows clear differentiation of samples; for example, from the east or west coasts of the United States or from the United States, Europe, or the Middle East. PCA and PLSDA models for the east and west coast data sets were created and show significant separation between the sand originating from different geolocations. Raman spectra of the materials show that the main feature is SiO_2, but other spectral features originating from different oxides allow the differentiation of samples collected from the different locations. Data fusion technology was applied to the set of XRF and Raman data to create PCA and PLSDA models. Misclassification in PLSDA models was studied using randomly selected samples from available data. The results of standard and data-fused analysis are compared and discussed. In conclusion, this study provides methods that allow the differentiation of samples without knowing the actual concentration of elements/oxides and, therefore, this approach does not require any calibration for material identification.

Sand, Glass, XRF

B85 Differential Sampling of Footwear to Separate Alternative Particle Signals

David A. Stoney, PhD, Stoney Forensic, Inc, 14101-G Willard Road, Chantilly, VA 20151; and Paul L. Stoney, MBA, 14101-G Willard Road, Chantilly, VA 20124*

After attending this presentation, attendees will understand the method and potential contribution of the differential sampling of footwear as a means of separating sets of small particles that have accumulated as a result of exposures to different environments at different times.

This presentation will impact the forensic science community by providing an efficient, practical method to separate loosely, moderately, and tightly held particles from the surfaces of footwear. The inter-comparison and subtraction of these different particle sets will provide a means to separate alternative particle "signals" (as, for example, when subtracting a background spectrum in Infrared (IR) spectroscopy).

The separation of particle signals arising from different sources is one of the enabling operations for Particle Combination Analysis (PCA).[1] Although it is well recognized that criminals track dusts to and from every crime scene, dust particles on a suspect's shoes are very seldom used as evidence linking the accused to the crime. The major obstacle preventing the use of this type of evidence is that the shoes have mixtures of particles arising from activity before, during, and after the crime itself.[2] Methods separating the evidentiary particle "signal" from background noise would enable a powerful new and widely applicable forensic capability. This capability would augment traditional footwear pattern evidence with objective quantitative associations, addressing one of the specific issues raised in the 2009 National Academy of Sciences (NAS) Report, Stregnthening Forensic Science in the United States – A Path Forward. To help pursue this possibility, methods have been developed and tested that employ a series of successively more aggressive particle sampling protocols to separate loosely held, moderately held, and tightly held particles from the surfaces of footwear.

Two distinctly different and commonly encountered types of shoe soles were used in this study: athletic shoes (with flexible rubber soles) and work boots (with hard rubber soles). Eighteen new pairs of shoes of each type were sequentially exposed by walking a distance of 250m in three outdoor environments. Environments were evaluated and selected based on a well-defined accessible path and the presence of particle populations that were: (1) individually diverse; and, (2) collectively distinguishable from one another by defined qualitative and quantitative particle characteristics. Separate pairs of shoes were used for single-environment exposures (12 pairs, 6 of each type, 2 for each of the 3 environments) and sequential exposures to all three environments (24 pairs, 12 of each type, 2 for each of the 6 alternative sequences). Following exposures, particles from the outermost surfaces of the footwear soles were harvested by three progressively more aggressive methods: walking on paper (12 steps on butcher paper); electrostatic lifting (six lifts using a commercially available electrostatic lifter); and surface swabbing. Swabbing was conducted using lint-free clean room swabs moistened with 2% pre-filtered aqueous ethanol, resulting in particle suspensions. Particles on the paper and electrostatic lifts were collected using the same swabbing procedure, resulting in similar particle suspensions. Particle suspensions were fractionated by sieving and settling to recover fine sand and silt-sized particles. Particle types and quantities were measured by point counting using polarized light microscopy.

For each of the pairs of shoes, and for each of the successively more aggressive sampling methods, fine sand and silt-sized particles were recovered in sufficient quantities for quantitative analysis and comparison of soil minerals by polarized light microscopy (n >300). Under the experimental conditions, athletic shoes (with flexible rubber soles) retained more fine sand and silt-sized particles than did the work boots (with hard rubber soles).

The successful differential sampling of particles adhering to footwear surfaces enables the comparison of particle populations that adhere with different tenacities, allowing the development and exploitation of methods that can recognize different particle signals occurring simultaneously on these surfaces. Differential sampling also allows more fundamental investigations of phenomena contributing to the transfer and persistence of small particles on footwear.

This project was supported in part by an award from the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect those of the Department of Justice.

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Trace Evidence, Footwear, Particle Signals

B86 Total Imaging Analysis of Paint

Roger Kahn, PhD, Harris County Institute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054; and William M. Davis, PhD, 1885 Old Spanish Trail, Houston, TX 77573*

After attending this presentation, attendees will understand a new approach to the analysis of paint samples (or any lamellar exemplars).

This presentation will impact the forensic science community by demonstrating an alternative approach to paint analysis which may then become available in additional laboratories.

The analysis of paint evidence has a long history as a subdiscipline of trace evidence. Automotive paint, in particular, can be probative in hit-and-run cases. These paints are analyzed for their class properties and can be cross-referenced to databases such as Paint Data Query (PDQ). Routine paint analysis involves optical characterization of component layers followed by chemical analyses of those layers via vibrational spectroscopy (Infrared (IR) and Raman), pyrolysis gas chromatography, Scanning Electron Microscopy with Energy Dispersive X-ray (SEM/EDX) analysis, and Laser-Induced Breakdown Spectroscopy (LIBS), to name a few. Many of these are non-destructive, inducing little damage to original evidence.

Recent advances in IR imaging provides the paint examiner with a new tool. Particularly useful are instruments that have the capability to capture microscopic IR images with a Total Attenuated Reflection (ATR) device. These devices allow for better energy throughput to the sample than traditional IR microscopes. It should be noted that all of these devices collect data using interferometers (Fourier Transform). IR techniques alone assist in the identification of the majority of organic components of binders and extenders as well as some inorganic components. Inorganic components not captured with IR may be found in an X-ray analysis using SEM/EDX.

Traditionally layer characterizations (e.g., number of layers, colors, and dimensions) are performed by optical microscopy. Difficulties may arise when adjacent layers appear as the same color and cannot be observed with visible light, whether polarized or not. These layers may become apparent when using various light sources, although these manipulations tend to be subjective in nature and prone to artifact. Alternative contrast sources have been shown to help develop the number of layers in a paint sample, one of which is backscatter electron detection in an SEM.

Results will be presented of a paint analysis scheme which not only takes into account the visible images but images in the IR and X-ray regions. One acrylic mounted paint chip can be used throughout the experimental process. The IR images are color-enhanced representations of IR space in which each pixel represents an IR spectrum in the energy range of $4{,}000^{-1}$-750 cm^{-1}. X-ray maps of constituent elements are provided for each sample alongside a back-scatter electron detection image of the lamellae. The maps are acquired using a variable pressure option on an SEM and hence no sample preparation is required to avoid charging of the sample and image degradation over the course of the map acquisition. Validation samples will be provided that show structural and spectroscopic replication of known PDQ samples.

The techniques discussed in this presentation may be extended to other lamellar materials such as food packaging.

Paint, FTIR Imaging, X-Ray Mapping

B87 When Are Variations in Duct Tape the Result of True Differences? A Cautionary Tale

Diana M. Wright, PhD, FBI Laboratory Division, Chemistry Unit, Rm 4220, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, attendees will understand two scenarios in which observed variations between duct tape samples were evaluated independently and produced different conclusions regarding an association. This information may be helpful in interpretation of a comparative duct tape analysis and in assessing the significance of those results.

This presentation will impact the forensic science community by increasing knowledge of the variation that can occur between mass-produced materials, specifically those manufactured by the same producer using similar technologies. The effect these results may have on association/discrimination criteria will be discussed.

Duct tapes are a type of trace evidence commonly encountered in North American forensic laboratories. Techniques used to analyze duct tapes can readily discriminate different products, even those from a single manufacturer. Even minor physical (e.g., backing film thickness) or compositional (e.g., intensity variation in filler components) differences may be justification to discriminate tapes.

In this presentation, differences observed in the Fourier Transform Infrared (FTIR) spectroscopy data of duct tape samples manufactured by the same company will be discussed.

In the first example, results of a duct tape homogeneity study will be presented in which samples taken from different sections of the same 60-yard roll were determined to be statistically different; however, these differences were not considered to be significant when spectral overlays were evaluated by a trained forensic examiner. Rather, it was determined that sample thickness differences were likely to be the cause of the differences that were reported by the statistical analysis.

The second example arose from a commercially available proficiency test in which an adhesive residue sample was compared to two partial rolls of duct tape. According to information later obtained from the test provider and the tape manufacturer, these rolls were different products manufactured by the same company but marketed to different do-it-yourself home goods stores using different labeling. The rolls were readily distinguishable by film thickness, a parameter not able to be assessed for the adhesive residue. Therefore, the examiner compared the residue to both rolls using the analytical suite routinely employed for duct tape comparisons: physical characteristics, FTIR, Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS), and X-Ray Diffraction (XRD). Based on this testing, the adhesive composition of both rolls and the adhesive residue sample were concluded to be analytically indistinguishable by the examiner. Minor intensity differences in the FTIR data between the two rolls were noted and attributed to possible sample thickness variation in sampling. After the results were submitted, the test provider reported that the residue was attributable to only one of the submitted rolls.

The examiner submitted the test samples to the tape manufacturer for analysis and assessment. Subsequent discussions indicated that the observed intensity differences may have been the result of a different processing method, which could explain the differences in the FTIR data as true concentration differences.

It is anticipated that the results of this study might serve as a cautionary tale regarding the interpretation of any possible differences in manufactured, mass-produced materials. This discussion illustrates the need to better define the association/discrimination criteria that should be used in comparative duct tape analyses. The samples discussed in these examples were all pristine cuttings from new rolls of duct tape. Evidentiary duct tape samples have varying conditions and degrees of cleanliness, which would further factor into assessing the significance of any possible differences.

The language used to report the association/false inclusion will be provided to further illustrate how the use of qualifying statements, interpretation models, and justification for the strength of an examiner's reported opinions are necessary and beneficial to the process of interpreting a comparative analysis report.

Duct Tape, Variation, Interpretation

B88 Intra-Roll and Intra-Product Variations in Duct Tapes

Andria H. Mehltretter, MSFS, FBI Laboratory, Chemistry Unit, 2501 Investigation Parkway, Quantico, VA 22135; Diana M. Wright, PhD, FBI Laboratory Division, Chemistry Unit, Rm 4220, 2501 Investigation Parkway, Quantico, VA 22135; and Michael A. Smith, PhD, 2501 Investigation Parkway, Chemistry Unit, Quantico, VA 22135*

After attending this presentation, attendees will better understand the variation that can be expected within a single roll of duct tape as well as between rolls of those same tape products over time. This information may be used to better interpret the results of a comparative duct tape analysis and to assess the significance of those results.

This presentation will impact the forensic science community by increasing the knowledge of duct tape products, specifically the variation that can be observed/measured between tape products over an approximate six-month time frame (i.e., batch-level differences). The effect these results may have on association/discrimination criteria will be discussed.

Duct tapes are a type of trace evidence commonly encountered in North American forensic laboratories. Historically, the forensic community has relied on empirical observations, discussions with manufacturing representatives, and limited research studies to learn how and to what extent duct tape products vary. In order to provide a more robust understanding of duct tape variation, a two-part comprehensive study was devised.

Part 1 of this study examined how individual rolls of duct tape vary along both an individual roll's length as well as across a jumbo roll of duct tape. The jumbo roll analysis provided an evaluation of different rolls of the same product manufactured at the same point in time. It was determined that scrim count, width, thickness, and adhesive composition vary only to a limited extent along the length of an individual roll of tape. Further, aside from width, minimal variation in these characteristics occurs between different rolls cut from the same jumbo roll. Statistical analysis of the thickness and adhesive composition via Fourier Transform Infrared (FTIR) spectroscopy indicated that some statistically significant differences could be observed; however, these differences were minor and not meaningful in the context of the forensic examination of mass-produced products.

Following publication of those results, Part 2 of this study was undertaken in which the same manufacturers provided additional sample rolls of their most common products over an approximate six-month timeframe. Based on the results of Part 1, scrim count, width, thickness (both overall and film only), and adhesive composition (via FTIR) were further evaluated. Specifically, scrim count and width were measured to see whether any variation could be detected along the length of an individual roll, between rolls cut from the same jumbo roll, or between rolls produced at different points in time. Additionally, thickness and adhesive composition were evaluated to assess whether differences could be detected in these features between rolls produced at different times. When appropriate, multivariate statistical analyses were conducted on the data.

It is anticipated that the results of this study might be used to better define the association/discrimination criteria that should be used in comparative duct tape analyses. Further, an understanding of the variation over time can be used to determine whether forensic examiners can detect batch-level differences, which is important for interpreting the significance of an inability-to-discriminate result.

Duct Tape, Variation, Interpretation

B89 Hair Analysis: Learning From the Past and Moving Toward the Future

Sandra Koch, MS, Penn State University, Dept of Anthropology, Carpenter Bldg, Rm 403C, University Park, PA 16802*

After attending this presentation, attendees will better understand how the existing nomenclature used in microscopical hair analysis originated, the pressing need to revise how hairs are characterized for ancestry and root growth stage, and the significance of differences in microscopic characteristics present within hair structures.

This presentation will impact the forensic science community by emphasizing the need to standardize terminology and to promote an approach for microscopical hair examinations that is inclusive to the various disciplines studying hair today.

Microscopical and morphological analysis of hair occurred originally in anthropological studies of population groups in which racial classification was a primary concern. The methods and terminology used were then imported into medicolegal contexts and cosmetological research. As each field developed their unique analyses of hair, communication between fields declined. One of the unfortunate side effects was the continued use of outdated racial terminology in forensic hair examinations long after the terminology had been abandoned in anthropology. The more acceptable terminology currently being used outside of forensic hair examinations will be described with the goal of changing racial descriptors to ancestry-based groupings.

Correctly differentiating the growth stages of hair roots based on morphological characteristics and the presence of associated follicular tissue is important in forensic hair examination for determining whether a hair may be suitable for nuclear DNA testing or mitochondrial DNA. Unfortunately, different scientists look at an evidential hair and often differ in their classifications of root growth stage. This happens irrespective of experience so discussion will include the different growth stages, how they can be reliably differentiated, what examinations are most appropriate for these determinations, and the effects of conflicting growth stage categorization on forensic research.

Recent reviews of past hair examinations have focused on how laboratory results have been reported and used as the focus for testimony. Such reviews often have a positive outcome because they lead to improvements in how the discipline presents its findings and limitations. The various resources available through the Scientific Working Group for Materials Analysis (SWGMAT) and the Organization of Scientific Area Committees (OSAC) Materials (Trace) Subcommittee will be discussed, as well as certification, training, and research needs.

The field of hair analysis is in a state of transition, but with a careful review of the language used and what it means to others, a transparent evaluation of the evidence and the scientific basis for the results can be presented. With an interdisciplinary outlook, the field can more easily embrace change both in expression of results but also in application of new technology. By moving away from isolationist practices and research, hair analysis will continue to improve and remain relevant in forensic laboratories and research for years to come.

Hair, Telogen Roots, Ancestry

B90 Introducing New Instrumental Technologies in the Forensic Drug Laboratory — Learning From Past Experiences

Sandra E. Rodriguez-Cruz, PhD, Drug Enforcement Admin, Southwest Laboratory, 2815 Scott Street, Vista, CA 92081*

After attending this presentation, attendees will better understand the difficulties encountered when introducing new technologies into the laboratory.

This presentation will impact the forensic science community by presenting and discussing numerous factors affecting the introduction of new technologies into the laboratory and how those difficulties can be overcome by learning from past experiences.

Forensic laboratories responsible for the analysis of seized drugs must be equipped with the scientific infrastructure necessary for the qualitative and quantitative analysis of these materials. Minimum standards for the analysis of seized drugs have been published by the Scientific Group for the Analysis of Seized Drugs (SWGDRUG Recommendations Version 7.0; www.swgdrug.org). SWGDRUG recommends that laboratories implement analytical schemes involving the combination of Categories A, B, or C techniques, classified as such based of their maximum degree of discrimination. Depending on their legal jurisdiction, laboratories throughout the United States and abroad should implement analytical schemes to fulfill these minimum recommendations while providing the law enforcement and legal communities with accurate and timely results.

Depending on their analytical scheme and the suspected drug under investigation, analysts have the choice of utilizing a wide array of chemical and instrumental test procedures. Color and microcrystalline tests provide presumptive information, while instrumental techniques like Gas Chromatography/Mass Spectrometry (GC/MS) and Infrared (IR) spectrophotometry have become mainstays in the forensic laboratory because of their ability to provide structural information for a compound and, for most cases, a final identification. But these analytical techniques, although proven robust, do have limitations on their applicability.

New technologies are developed every few years that can improve the quality of forensic analyses and also potentially decrease turnaround times for results. Research and pharmaceutical laboratories are often the primary users of such new technologies, a result of the private nature of these industries, as opposed to the government-funded crime laboratories. With high caseloads and the continual appearance of new designer drugs, the need to introduce and establish these technologies in the forensic laboratory is essential; however, the introduction of new products and instrumentation sometimes proves to be a difficult task. This presentation will discuss some of the challenges faced when introducing these technologies into the crime laboratory. Examples of new technologies (Liquid Chromatography/Mass Spectrometry (LC/MS), Ultra High-Performance Liquid Chromatography (UHPLC), and Raman spectroscopy) will be discussed.

Some of the factors hindering the use and establishment of new analytical techniques in the seized-drug laboratory are obvious. The most often cited are lack of funding sources and the complacency among laboratory personnel; that is, the unwillingness to "try something new." Other factors are not often discussed, but they play equally important roles in the matter. Managers and analysts need to work together, be willing and comfortable about changing their "routine ways," especially when the alternative new technology can provide a higher-quality result in a faster manner. Even when laboratory personnel are willing to look into new technology, large backlogs and the lack of expertise may hinder their progress. Furthermore, managers and analysts are often overwhelmed by the information provided by instrument vendors, and quality assurance requirements and procedures are not always considered during vendor-laboratory discussions. Many times this results in laboratories acquiring instrumentation with much higher capabilities (and cost) than they realistically need. And finally, but certainly not less important, are the lack of training opportunities for analysts willing to use the new technology.

Criminalistics, New Technologies, Mass Spectrometry

B91 Embracing Change: Transitioning Pattern Evidence Research Into Forensic Science Operations

JoAnn Buscaglia, PhD, FBI Laboratory, CFSRU, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, attendees will understand the essential factors for the successful transition of pattern evidence research into operational practice.

This presentation will impact the forensic science community by describing the key factors for insuring the successful transition of pattern evidence research into practice, as well as some of the impediments to such transition. Understanding the factors for successful transition will help forensic practitioners rapidly adopt new technology and will enhance the accuracy and reliability of forensic science.

Forensic science research and development efforts have seen a significant rise over the past decade, which may be attributed to many factors. The increased lay knowledge of forensic science and the "CSI effect" resulted in expectations of high-tech methods as "common practice" in forensic science; more universities began offering forensic science coursework and degree programs, which in turn focused more research attention on forensic science issues. The 2009 National Research Council of the National Academy of Sciences (NAS) Report, Strengthening Forensic Science in the United States – A Path Forward, which questioned the accuracy and reliability of some of the oldest and most renowned forensic science disciplines, specifically called for research to assess the scientific foundations of the pattern evidence disciplines.[1] Finally, more federal research funds became available for both basic and applied research in forensic science disciplines in light of the 2009 NAS Report.

However, increases in funding and research effort do not always yield a significant increase in useful products transitioned into operational practice. It is important to note that only a small fraction of research and development in most scientific endeavors ever reach successful completion, and an even smaller percentage are put into practice. With the forensic sciences receiving increased scrutiny as well as increased research resources, it is more important than ever to insure the successful transition of research products into the operational forensic laboratory.

Lessons learned from the transition of pattern evidence research into operational practice will be presented. Factors contributing to successful implementation as well as impediments to transition will be discussed using examples from research in latent prints and questioned documents. Examples of research transitions will include small, incremental changes to methods and practice, adoption of novel analytical methods, and research that provided foundational support for continued practice or significantly changed the analysis or interpretation of the discipline.

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1. National Research Council, National Academy of Sciences (2009) *Strengthening Forensic Science in the United States: A Path Forward* (National Academies Press, Washington, DC).

Research, Pattern Evidence, Criminalistics

B92 Implementing 3D Technology Into a 2D Philosophy

Heather J. Seubert, MS, FBI Laboratory, Firearms/Toolmarks Unit, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, attendees will better understand some of the implementation hurdles that can be easily overcome for introducing new technologies and developing a strategy as to how to prepare for implementation.

This presentation will impact the forensic science community by providing an overview of integrating new technology into a science that hasn't seen much change in its methodology. This presentation will also inform the forensic science community about emerging technologies within comparative-based disciplines that are being evaluated for future casework applications.

The firearms/tool marks discipline can be traced back to the early days of the 1920s when early pioneers such as J.H Mathews, Calvin Goddard, and James Hatcher were exploring "forensic ballistics." In those early days of researching, many of their methods showed close parallels to the new technologies that are emerging relying upon measurements and illumination techniques. Also, the same question continues to motivate the practitioner to determine whether or not a bullet or cartridge case could be "identified" to a particular firearm. Even before the arrival of the comparison microscope, a match could be determined through the use of a filar micrometer, which was simply a special device placed at the top of a compound microscope and contained a scale and a cross hair, which moved along a scale. Another method used was the method of interchange. This method depended upon an illumination technique which involved a long camera set-up with a short lens.

Now, nearly 90 years later, these very principles of measuring and illumination that highlighted areas on bullets and cartridge cases then are being advanced to a level beyond the 2D world. These advancing technologies require the right approach for implementation and almost a strategy map for integration. The Firearms/Toolmarks Unit (FTU) of the FBI Laboratory has been evaluating and validating 3D technologies to enhance an identification conclusion with the desire to establish a qualitative and quantitative threshold which will introduce an objective component to this subjective discipline. Over the past two years, the FTU has acquired many of the advancing technologies available to the forensic science community in an effort to develop methodologies, build collaboration, and further the discipline of firearms and tool marks. The technologies the FTU has begun evaluating includes the Sensofar Confocal Microscope, the TopMatch Gelsight technology, the Alicona Focus Variation, and the EvoFinder. Over the course of this validation journey, the FTU has experienced challenges. This presentation will highlight some of those challenges (such as gaining acceptance from practicing examiners, fiscal forecasting for support from management, surviving technology hurdles) and will discuss how to prepare personnel to perform the evaluation and testing of these systems, setting up organized sample databases, preparing samples for testing, maintaining company support, estimating the fiscal projections for the continual maintenance and upgrades, building collaboration, and looking downstream to how the results generated will be articulated in a report of examination. And, finally, this presentation will examine how the community prepares for the legal challenges that will accompany the admission of these technologies in a court of law.

Validation, Legal Challenges, Technology

B93 The Transition From Research to Routine Use in the Forensic Chemistry Laboratory

Jose R. Almirall, PhD, Florida International University, Dept of Chemistry, University Park, Miami, FL 33199*

The goal of this presentation is to cover the scientific requirements, obstacles encountered, and collaborative spirit necessary to bring about the successful transition from the research laboratory to the routine operational laboratory.

This presentation will impact the forensic science community by assisting researchers to gain a perspective on the necessary operational requirements of forensic laboratories. Forensic laboratory personnel will learn what motivates researchers so both groups can better collaborate to bring research into routine usage.

The chemical analysis and comparison of a number of solid matrices of interest to forensic scientists are made possible by progression from fundamental research and method development, validation in several forensic laboratories, and the publication of international standards. The transition of high-sensitivity elemental analysis of materials, including glass from the research laboratory to adoption and routine use in a forensic laboratory is a slow and laborious process. This presentation will cover the scientific requirements, obstacles encountered, and collaborative spirit necessary to bring about the successful transition. Laser Ablation-Inductively Coupled Plasma/Mass Spectrometry (LA-ICP/MS) has been called the "gold" standard for solid-sampling and high-sensitivity elemental characterization of materials providing sub-ppm detection limits of elemental analytes encompassing almost 70% of the periodic table. In addition, LA-ICP/MS provides true *quantitative analysis* data that can be used in numerical/statistical hypothesis testing to determine "match" and also to populate databases that are useful to determine the probability of a match for a given elemental profile. Several forensic laboratories have collaborated on method development and optimization as reported by the European Union-funded Natural Isotopes and Trace Elements in Criminalistics and Environmental Forensics (NITECRIME) effort (2000-2005) and continued by the National Institute of Justice (NIJ) -funded Elemental Analysis Working Group (EAWG) effort (2008-2012).[1,2] The interlaboratory trials eventually resulted in high-quality performance of these methods for the analysis of glass in forensic laboratories and this effort culminated in the publication of international (American Society for Testing and Materials (ASTM)) analytical consensus standards for the examination of these materials.[3,4] More than 30 forensic laboratories around the world now routinely employ the use of LA-ICP/MS for materials characterization on every continent and the history of elemental analysis provides a good model on how forensic method development should progress from basic research to routine use and acceptance in the courtroom.

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2. Trejos T., Koons R., Becker S., Berman T., Buscaglia J., Duecking M., Eckert-Lumsdon T., Ernst T., Hanlon C., Heydon A., Mooney K., Nelson R., Olsson K., Palenik C., Pollock E., Rudell D., Ryland S., Tarifa A., Valadez M., Weis P, Almirall J.R. Cross-validation and evaluation of the performance of methods for the elemental analysis of forensic glass by μ-XRF, ICP-MS and LA-ICP-MS, *Anal. Bioanal. Chem.*, 2013, DOI 10.1007/s00216-013-6978-y.
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Basic Research, LA-ICP/MS, Standardization

B94 The Future of Forensic Instrumental Methods of Analysis

Glen P. Jackson, PhD, West Virginia University, Dept of Forensic and Investigative Science, 208 Oglebay Hall, Morgantown, WV 26506-6121*

After attending this presentation, attendees will understand the current state-of-the art in instrumental methods of analysis and where the trends are likely to lead. Attendees will also gain an appreciation for factors that are likely to affect future trends.

This presentation will impact the forensic science community by providing practitioners with examples of technologies that have the ability to impact the way forensic analyses are performed. The possibility of performing on-site confirmatory tests has the ability to revolutionize the way forensic evidence is used and could result in criminal investigations being quicker and more efficient.

Instrumental methods of chemical analysis provide objective and largely irrefutable circumstantial evidence in the support of criminal prosecutions. Chemical measurements can also be archived for validation or cross-examination by third-party expert witnesses and are widely accepted as among the most reliable of forensic techniques; they received no criticism in the highly critical 2009 National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States — A Path Forward*. Several recent advancements in different technologies have resulted in a shift toward on-site and *in situ* chemical measurements, and the forensic community will have some important decisions to make regarding its future in this uncertain area.

This presentation will cover a variety of developments in instrumental methods of analysis that have the potential to impact the way forensic analyses are performed and will engage in discussion on the likely effects of their adoption into different levels of casework. For example, technologies can be incorporated at the crime scene or in the presence of suspects/victims, in remote vehicles, at booking, or in the crime laboratories. The point of analysis will influence the expertise of the instrument operator, the potential for contamination or mishandling, the relevance to evidence collection, and the relevance to the prosecutorial process. More specifically, the ability to identify substances in real time *in situ* can assist investigators with evidence collection and the prioritization of evidence. Similarly, the ability to identify suspected drugs before or during booking can assist with the accurate investigation of alleged crimes.

This presentation will also discuss some of the major concerns about the adoption of new technologies, including: (1) the expertise and ability of police officers to conduct chemical measurements; (2) the uncertainty or insecurity of laboratory-based drug analysts; and, (3) the ability of on-site measurements to withstand scrutiny in court.

Finally, this presentation will address some factors that are likely to influence the adoption of instrumental methods of analysis. These factors include the requirement for some brave game changers to set precedents with the adoption of new technologies and the requirement for a less conservative system or branch of practitioners who can adopt or propel such cutting-edge developments. The discussion of pros and cons of future technologies should not be construed as an endorsement or warning for change, but as an initiation for discussion as to how to prepare for this domain in the future of criminalistics.

Instrumentation, Analysis, *In Situ*

B95 Multi-Software Interpretation of Complex Mixture DNA Profiles: A Comprehensive Approach to Explaining DNA Interpretation Results in Courtrooms

Eugenio Alladio, MS, Centro Regionale Antidoping "A. Bertinaria," Regione Gonzole 10/1, Orbassano (Turin) 10043, ITALY; Paolo Garofano, MD, PhD*, Accademia Italiana di Scienze Forensi, via Manlio di Veroli, 3, Rome 00199, ITALY; Roberto Testi, MD, PhD, ASL Torino 2, Via Lessona 54/12, Torino 10145, ITALY; Marco Vincenti, MS, Centro Regionale Antidoping, Regione Gonzole 10/1, Orbassano, Torino 10043, ITALY; Denise Caneparo, MS, Regione Gonzole 10/1, Orbassano, ITALY; and Giuseppina D'amico, Regione Gonzole 10/1, Orbassano, ITALY*

After attending this presentation, attendees will understand how to use a multi-software probabilistic approach for Low Template DNA (LT DNA) mixtures in highly challenging samples to better explain evidence in court, avoiding expert discussion when using different interpretation strategies.

This presentation will impact the forensic science community by illustrating how diverse the results of different software can be and how to manage these results by providing the most conservative and reproducible data in order to deliver complete statistical information.

The goal of this study was to define a rigorous approach to LT DNA mixture interpretation using multiple probabilistic software programs. Despite several recommendations having been proposed over the past years concerning the importance of evaluating several factors which may affect inclusion or exclusion hypotheses from prosecutor or defense, a rigorous approach has still not been properly defined in order to establish a "universally accepted" methodology. Moreover, this lack of regulation and guidelines leads experts to differently interpret evidence in courtrooms by applying several statistic approaches, which are often incomprehensible to the jury and legal experts. This practice causes the ability to make a judgement "beyond any reasonable doubt" even more difficult. In order to improve judgement capability and impartiality, this study adopted two models: the semi-continuous approach (using LRmix Studio and Lab Retriever software) and the fully continuous approach (using Charles Brenner DNA•VIEW™ mixture solution software).

Both models helped to highlight the difficulties encountered when evaluating challenging Short Tandem Repeat (STR) profiles and clarified the need for extreme caution in order to achieve a correct interpretation of the DNA evidence as this can heavily affect the outcome of a trial. After thorough validation studies, this research developed the "statistic consensus approach." In practice, this approach compares all Likelihood Ratio (LR) values obtained from all software used. If all LR results are similar and convergent, then the most conservative LR value obtained is reported. On the contrary, if LR results are not similar, the interpretation process provides an inconclusive decision. This approach resembles, in a complex way, the consensus method itself, which makes use of alleles observed in different replicates.[1-3] Due to this approach, it seems possible to conclude that certain suspects under investigation are unquestionable contributors to LT DNA mixtures under investigation. Even though the application of several software programs and different models is questioned by some scientists, this approach has already been successfully tried in court. The presentation of results were made easier during the trial since this approach considers different advocated issues such as the level of conservatism, the semi-continuous model's comprehensibility, and the fully continuous model's complexity.[4] Actual cases using this approach will be presented.

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1. Kokshoorn B., Blankers B.J. Response to Grisedale and Van Daal: comparison of STR profiling from low template DNA extracts with and without the consensus profiling method. *Investig. Genet.* 2013, 4:1.
2. Pfeifer C.M., Klein-Unseld R., Klintschar M., Wiegand P. Comparison of different interpretation strategies for low template DNA mixtures. *Forensic Sci. Int.* Genet 2012, 6:716–722.
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LT DNA Mixture Interpretation, Statistical Consensus Profile, Probabilistic Software

B96 Threshold to Probabilistic DNA Profile Interpretation: Why Change?

Stuart Cooper, MSc, ESR, Private Bag 92021, Auckland 1142, NEW ZEALAND; Catherine E. McGovern, MSc, ESR, Private Bag 92021, Auckland 1025, NEW ZEALAND; Jo-Anne Bright, ESR Ltd, Hampstead Road, Auckland, NEW ZEALAND; Duncan Taylor, PhD, Forensic Science South Australia, 21 Divett Place, Adelaide 5000, AUSTRALIA; Damien Abarno, PhD, Forensic Science South Australia, 21 Divett Place, Adelaide 5000, AUSTRALIA; and John S. Buckleton, PhD, PB 92021, Auckland, NEW ZEALAND*

After attending this presentation, attendees will have expanded their knowledge base in relation to advances in DNA profile interpretation, particularly in the area of probabilistic interpretation methods. This presentation is intended to address the question: Why change from a binary/threshold-based method?

This presentation will impact the forensic science community by providing an overview of the changing science behind DNA profile interpretation and re-addressing the known shortcomings of current interpretation methods. The use of actual casework examples will help facilitate the transfer of knowledge.

There have been a number of recent reviews of laboratory practice that have resulted in negative media reports. These have led to some laboratories questioning their interpretation protocols and whether these are still fit for the purpose. One such review occurred in Australia in 2009 and resulted in the implementation of an Australasian consensus approach to profile interpretation. Since then, uptake of probabilistic software in casework has increased significantly around the world with many more laboratories currently investigating the move away from traditional threshold/binary based or combined probability of inclusion models.

A number of fully continuous probabilistic methods have been described that model allelic and stutter peak heights within a DNA profile. Such models can help address the known shortcomings of traditional methods of profile interpretation, such as those within a binary model.[1-3]

So, why change? This presentation will look back at traditional DNA profile interpretation methods and highlight known shortcomings. The use of probabilistic models and software will be shown to address these issues. This will demonstrate the power of such models and their ability to decrease rates of false inclusion and false exclusion for a range of profile types. In addition, results of previous studies demonstrating how the use of probabilistic software can, under certain circumstances, help facilitate a consistent approach to the interpretation and reporting of statistical findings in a given case will be provided.[4]

Actual casework examples will be provided, demonstrating the benefits which would not have been realized without the use of probabilistic software. These examples will hopefully allow attendees to visualize the advantages for actual casework samples and put the use of such models into context within the criminal justice system.

This presentation will conclude with a discussion of possible future directions for probabilistic software and the further benefits which this may yield.[5]

Reference(s):

1. Perlin M.W., Legler M.M., Spencer C.E., Smith J.L., Allan W.P., Belrose J.L., et al., Validating TrueAllele® DNA mixture interpretation. *J. Forensic Science*. 2011; 56:1430–1447.
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Probabilstic Interpretation, Continuous Models, Casework Examples

B97 Questioning the Unquestioned — Rethinking and Rejecting Traditional Mixture Concepts and Assumptions

Charles H. Brenner, PhD, 6801 Thornhill Drive, Oakland, CA 94611-1336*

After attending this presentation, attendees will appreciate that "contributor," "number of contributors," "allele present," "stochastic threshold," and other traditional mixture concepts are vague and not well-defined and, therefore, are not a solid foundation for mixture analysis.

This presentation will impact the forensic science community by highlighting pitfalls that are common to many or most mixture methods and which sometimes invalidate the results. Some remedies will be presented.

"Continuous" DNA mixture analysis is a big and welcome change from binary and other earlier methods, and with good reason it might be called a revolution or paradigm shift. A "mixture model" means a simplified description of DNA mixtures. A binary mixture model explicitly treats the mixture data as having just one dimension — for Short Tandem Repeat (STR) loci, it is the allelic repeat numbers. Phenomena such as dropout and stutter fit into the model awkwardly at best as ad hoc add-ons. A continuous mixture model acknowledges a full-fledged second dimension, namely the signal intensity or Relative Fluorescence Units (RFU) of each amplified allele. The extra ("continuous") dimension is modeled as a template amount randomly modified by the combination of amplification stochastic variation and injection sampling variation. Two dimensions instead of one is in an obvious sense a complication, but it also leads to simplification because it lets analysts model both dropout and most drop-in, as well as variations in peak height and stutter ratios in a coherent fashion simply as aspects of the same random variation rather than as multiple black boxes.

Like most changes, the continuous approach has arrived gradually through various intermediate stages such as "semi-continuous" methods and ad hoc ways to cater to possible dropout. Consequently, there was no dramatic moment at which it became obvious to re-evaluate the original assumptions. Now is a good time to question some of the unquestioned principles and concepts about mixture analysis, and doing so unearths some surprises.

"Exclusion," an ill-applied term, has been known as a charlatan for awhile. A suspect is non-excluded by one meaning of the word, then an exclusion probability is calculated using a different meaning. "Number of contributors" isn't a definable number in any practical sense, yet traditionally some mixture protocols wrongly instruct the analyst to begin by determining it. Furthermore, it need not be the same number for prosecution and defense, contrary to typical mixture protocols and software. Realistic examples will illustrate and explain substitute concepts, such as "degree of contribution," that make more sense.

Thresholds rarely have a natural role in the real world; "stochastic threshold" is a particularly unfortunate example as it was invented specifically for the analysis of *simple* stains and was borrowed even into binary mixture analysis only by mistake. In a continuous mixture model, it is natural to view all peak height ratios as more/less probable rather than yes/no possible. Hence, correcting the early mistake and banishing the "stochastic threshold" concept is a natural, though not inevitable, consequence of adopting a continuous mixture model.

In several ways, the continuous approach to mixture modeling tends to eliminate complication and encourages clear and explicit thinking about the analysis process; however, there are various ways to devise a continuous model and retaining some of the traditional misconceptions is possible. Critical analysis should begin with writing down the model. If that is done clearly, then assumptions can be better recognized and it will be easier to see misconceptions and know where to be wary.

Mixture, Pitfall, Continuous

B98 Demystifying Mixture Interpretation Software Tools (MIST) — Practical Applications and Implementation Strategies for DNA MIST

Patricia A. Foley-Melton, PhD, RTI International Center for Forensic Science, 3040 E Cornwallis Road, Bldg 3, Rm 201, Research Triangle Park, NC 27709; Jeri D. Ropero-Miller, PhD, RTI International, 3040 Cornwallis Road, PO Box 12194, Bldg 7, Rm 211, Research Triangle Park, NC 27709; and Lyndsie N. Ferrara, MS, Duquesne University, 600 Forbes Avenue, Pittsburgh, PA 15219*

The goals of this presentation are to: (1) investigate how DNA MIST have been used for crime laboratory applications; (2) provide considerations from current users to inform potential technology adopters; and, (3) assist with implementation planning by providing practical and technical considerations through examples of real-world applications.

This presentation will impact the forensic science community by informing forensic practitioners in crime laboratories of the potential benefits of these tools and supporting the decision-making process for adoption of this technology into forensic DNA crime laboratories.

This presentation will provide an overview of the reported landscape study of DNA mixture interpretation software tools conducted by the National Institute of Justice's Forensic Technology Center of Excellence at RTI International with support from Duquesne University.

Many crime laboratories have recognized the benefits of adopting a DNA mixture interpretation software tool that assists with the challenges of complex mixture interpretation and provides statistical analysis. Forensic DNA crime laboratories, therefore, benefit from a study that reviews current product offerings, features, and capabilities, and examines how this technology is chosen, acquired, implemented, and validated. Although probabilistic software tools can assist with many of the issues experienced with complex mixtures, it is important for laboratories to understand the features and limitations of the different tools and make a choice that best meets their needs.

The objectives of this study were to investigate to what extent DNA mixture interpretation software tools have been used for DNA forensic crime laboratory applications, provide considerations from current users to inform potential technology adopters, and assist with implementation planning by providing practical and technical considerations through examples of real-world applications. This study captures the current state of DNA mixture interpretation software tools and the potential benefits of adoption. The considerations and benefits described in this study were obtained through interview discussions with subject matter experts, including crime laboratory practitioners, stakeholders, technology developers, academics, and key decision makers. The captured discussions highlight the different needs and methods for procurement, validation, and implementation. This study also contains a comprehensive review of secondary sources, such as journal and industry literature, for information related to need, successful use, developmental validation, and adoption criteria. In addition, the key considerations for successful implementation are discussed, including comprehensive training on the software and the underlying mathematical model, the resources needed for internal validation, including planning, labor, and time, and the potential for additional support from the laboratories' Information Technology (IT) departments.

With the continued increase of complex mixture data, the need for mixture interpretation software tools is of growing importance to the forensic DNA community. Given the availability of open-source and commercial software options, forensic DNA crime laboratories must assess their needs to find the most suitable software tool.

DNA, Mixture, Software

B99 A Hybrid Machine Learning Approach (MLA) for DNA Mixture Interpretation

Michael Marciano, MS, Forensic and National Security Sciences Institute, Syracuse University, 1-014 Center for Science and Technology, Syracuse, NY 13244-4100; and Jonathan Adelman, MS*, Syracuse University, CST 1-014, 100 College Place, Syracuse, NY 13244*

After attending this presentation, attendees will better understand machine learning approaches and the potential impact of applying machine learning to aid in the interpretation of DNA mixtures.

This presentation will impact the forensic science community by introducing the novel approach of incorporating machine learning as an analytical tool for DNA mixture deconvolution. In addition, its utility will extend past the current capillary electrophoresis-based data to utilize sequence data. This approach will produce highly informative, reliable intelligence or investigative leads from complex DNA mixtures that currently cannot be interpreted via other methods.

The challenge of DNA mixture interpretation is at the core of forensic genetic identification. Mastery of this interpretation can significantly impact the course of criminal investigations and/or quality of intelligence. In forensic settings, scientists responsible for mixture interpretations have relied on data from empirical validation studies, computation, and experience. Limitations are inherent in human-based analyses while other deficiencies are specifically related to computational capacity complexity and time constraints. There are expansive data sets that may be computationally leveraged to better address DNA mixture deconvolution; a classification approach capable of extracting maximum information from those data sets may be better able to interpret complex mixtures.

An MLA is ideally suited to such complex data sets and can indeed be used in classification problems. Hybrid systems capable of combining "human-like" subjective reasoning with the computational power of artificial intelligence techniques may be of special interest. These MLAs may provide higher-confidence and more rapid and expanded deconvolution capabilities. The power in such a system stems from: (1) the ability of the algorithm to learn from an initial data set and subsequently classify mixtures from previously unseen data; and, (2) the influence from the human analyst's experience-derived "rule sets." The approach utilizes the strengths of both computationally intensive algorithms and expert systems. Candidate features such as peak height and ratios were identified for extraction from mixture data sets, and the subsequent evaluation will be used as the feature vector used as input for the machine learning algorithms.

Several MLAs will be evaluated, with training data drawn exclusively from mixtures of known contributors and proportions. Once trained and validated, the chosen MLA will have the potential to rapidly (within minutes) deconvolute increasingly complex mixtures of at least three contributors. The architecture of the MLA permits mixture analyses using diverse data types including DNA fragment data, Polymerase Chain Reaction (PCR) amplification parameters, and a wide array of instrument parameters. The data-agnostic structure will allow increased flexibility in adapting to analyses of new data types, such as next generation DNA sequence data.

DNA Mixture, Machine Learning, Deconvolution

B100 Separating DNA Mixtures by Computer to Identify and Convict a Serial Rapist

Mark W. Perlin, PhD, MD, Cybergenetics, 160 N Craig Street, Ste 210, Pittsburgh, PA 15213;and Garett Sugimoto, MS*, Kern Regional Crime Laboratory, 1300 18th Street, 4th Fl, Bakersfield, CA 93301*

After attending this presentation, attendees will understand some principles of using probabilistic genotyping separation of complex DNA mixtures to investigate serial crime, calculate match statistics, prepare for trial, establish genotyping reliability, and present computer results in court.

This presentation will impact the forensic science community by providing a case example of using sophisticated computer technology that resolves complex forensic evidence in order to simplify human understanding and the communication of statistical support.

In July and August of 2013, a masked man broke into four separate Bakersfield, CA, homes and assaulted nine women and children. Three women were raped and one child was molested. The police recovered biological evidence from three of the crime scenes.

The Kern Regional Crime Laboratory (KRCL) took 37 swabs and cuttings from garments, blankets, apartment surfaces, skin, body cavities, telephones, and zip ties. In October of 2013, KRCL tested these biological items with Identifiler® Plus to generate Short Tandem Repeat (STR) data. The items were largely low-level DNA mixtures of three or four people. Human review of this data was generally uninformative; however, one of the zip ties (found in the road outside a victim's apartment) was a DNA mixture with a clear major contributor. This major donor led to a Combined DNA Index System (CODIS) profile that identified gang member Billy Ray Johnson.

Mixture separation and comparison were performed on all 37 evidence items using TrueAllele® Casework probabilistic genotyping technology. The company and KRCL independently conducted this mixture interpretation, each using their own in-house computer.

In October, the company interpreted the 37 evidence items by computer, separating the mixtures into genotypes. Parallel processing permitted all the computer analyses to proceed simultaneously. Once the 11 reference samples became available, computer comparison between separated evidence and reference genotypes yielded match statistics that indicated inclusion or exclusion. The technology linked eight mixture items (purse strap, phone, phone cord, two pants, shirt, bathtub handle, and zip tie) to Johnson. The computer showed the mixtures also contained DNA from victims and other people, consistent with their statements. The company sent their case report on these findings to the Kern County District Attorney's office. A grand jury heard the DNA and other evidence in December of 2013. They indicted Johnson on 25 counts, and he was arrested.

Separately, the KRCL conducted an independent in-house TrueAllele analysis of the DNA mixture data. Their match statistics agreed with the company's results. The concordant inclusionary statistics ranged from hundreds to hundreds of quintillions, depending on DNA quantity.

At the 2015 trial, expert witnesses from the company and KRCL testified about their match results. Each group had independently calculated match statistics, arriving at the same conclusions regarding the individuals who had contributed their DNA to the crime scene evidence. The computer had dissected the mixtures to show who was (and wasn't) associated with each item.

The jury was informed about computer interpretation of DNA mixtures and viewed a PowerPoint® presentation that explained how a computer separates mixture data into contributor genotypes. Comparing a mixed evidence genotype that had been subjected to contributor separation by the computer with the reference genotypes, relative to a population genotype, visually clarified the match statistic. Using published validation studies, the expert witnesses established interpretation reliability and could give false positive rates for their statistical conclusions. The cross-examination was respectful of the science.

On April 21, a Bakersfield jury convicted Billy Ray Johnson of 24 (out of 26) felony charges. On May 19, the serial rapist was sentenced to life in prison without the possibility of parole plus 423 years. Computer technology had successfully analyzed otherwise "inconclusive" DNA evidence, helping to secure criminal justice and ensure public safety.

A case study will be presented in probabilistic genotyping as an example of the processing and presentation of complex mixture results. Familiarity with this approach will help criminalists obtain statistical results from low-level DNA mixtures containing three or more people and assist in explaining their findings in court.

DNA Mixture, Probabilistic Genotyping, Serial Rape

B101 Conceptual and Cultural Limitations Delaying the Transition to Probabilistic Genotyping in Forensic DNA Analysis

Mark R. Wilson, PhD, Western Carolina University, Dept of Chemistry/Physics, Forensic Science, Cullowhee, NC 28723*

After attending this presentation, attendees will better understand the current limitations affecting the transition to probabilistic genotyping in forensic DNA analysis.

This presentation will impact the forensic science community by providing an overview of current practices in Short Tandem Repeat (STR) mixture deconvolution and by contrasting some of these approaches to emerging methods.

The current culture of forensic science teaches us to think in binary (in/out or plus/minus) fashion. Often, the words that are used, including exclude, include, conclusive, inconclusive, match, identity, cannot exclude, etc., are treated as categorical absolutes rather than as proportionate, continuous, or semi-continuous concepts. This commonly accepted usage of terms within the field of forensic science does not serve us well in the real world of uncertainty and continuous variation, especially in cases where the evidentiary data are complex and difficult, if not impossible, to analyze without computer support. Due in part to training, it is difficult to throw away the binary method of thinking and fully embrace continuous reasoning — where certainty is correctly understood as applying within a context rather than absolutely — and uncertainty is embraced and measured rather than avoided.

Most approaches to STR mixture deconvolution have given at least some credence to a quantitative assessment of DNA mixture data, including those advocating thresholds and binary placement into categories; however, as time has passed, the binary approach has slowly been replaced by probabilistic reasoning that attempts to embrace data uncertainty rather than eschew it. So although the germs of these ideas have been present from the outset, they have only recently started to be fully appreciated.

All parties in this discussion agree that quantitative patterns are present in STR-based DNA mixtures, and that the goal of forensic interpretation should be to make full use of these data. Any deliberate restrictions imposed on the data will necessarily result in less than full use of the data. If potentially valuable information is discarded, then potential identification information is also lost, resulting in the potential for the DNA mixture to be considered as uninterpretable, which could result in the guilty remaining free and for cases to remain unresolved. Hence, justice is best served by utilizing the data in the most efficacious manner.

In fully probabilistic approaches to DNA mixture deconvolution, if the data itself are ambiguous, a properly validated computer program will express this ambiguity as uncertainty. This approach does not advocate for deliberate data rejection by applying thresholds that ignore data. If the data are uncertain, the program assigns less probability to particular outcomes, in effect spreading out, or diluting, the resulting probabilities of particular genotypes.

Fully probabilistic genotyping models are not constrained by thresholds and consider all of the genotype probabilities arising from the data. In order to estimate these probabilities, some approaches apply a hierarchical model with many integrated parameter variables, including peak heights, heterozygote peak imbalance, stutter, mixture weights, degradation, and noise.

Variance parameters in DNA mixture deconvolution can most accurately be estimated by using all relevant data, and ignoring relevant data necessarily reduces the accuracy of the estimates. This inevitably leads to higher uncertainty and less-definitive conclusions. Hence, there is no justification for deliberately discarding data, such as in the use of thresholds, nor is there any justification for invoking any "conservative" principles in STR mixture deconvolution, since once the appropriate variance parameters have been identified and modeled, a fully probabilistic approach provides a built-in protection from both overstated and understated conclusions.

A fully rational approach to mixture deconvolution holds that a high value of discrimination is always good. Importantly, efficient mixture deconvolution simultaneously affects both sensitivity and specificity and thus is good for both inclusions (sensitivity), and also for exclusions (specificity). The fully objective view places a great value on a wide separation between these two poles — because reducing uncertainty itself in forensic science is tremendously valuable.

This presentation will provide an overview of the conceptual and cultural limitations within forensic science to the adoption of probabilistic genotyping. Examples will be provided to emphasize the points covered.

DNA Mixtures, Genotyping, Probability

B102 Massively Parallel Sequencing — A Revolution for Complex Mixture Interpretation?

David Ballard, PhD, King's College London, 150 Stamford Street, London SE1 9NH, UNITED KINGDOM; Laurence A.E. Devesse, MA, King's College London, 4.124 Franklin Wilkins Bldg, 150 Stamford Street, London SE1 9NH, UNITED KINGDOM; Athina Vidaki, PhD, King's College London, 150 Stamford Street, Franklin Wilkins Bldg, London SE1 9NH, UNITED KINGDOM; Gabriella Mason-Buck, MSc, King's College London, Franklin Wilkins Bldg, 150 Stamford Street, London SE1 9NH, UNITED KINGDOM; and Denise Syndercombe Court, PhD, King's College London, 150 Stamford Street, London SE1 9NH, UNITED KINGDOM*

After attending this presentation, attendees will appreciate the potential that sequence analysis of Short Tandem Repeats (STRs) via massively parallel sequencing methodologies has to alter the way forensic science deals with complex DNA mixtures.

This presentation will impact the forensic science community by demonstrating that DNA mixtures currently believed to be of little evidential value may now be usefully analyzed due to the improvements in sequencing and statistical interpretation methodologies.

Complex DNA mixtures are often encountered during forensic casework; however, interpretation of these mixtures can be problematic and contentious. Advances achieved through novel software solutions underpinned by complex statistical methodologies have shown promise to improve this analysis. Further advances with the potential to substantially aid mixture interpretation are now available due to the enhanced ability with next generation sequencing/massively parallel sequencing to analyze not only the allele length but also the allele sequence and hence increase allelic discrimination.

The ForenSeq™ kit from Illumina® was released in mid-2015 and includes 58 autosomal, Y and X chromosome STRs along with 94 identity Single Nucleotide Polymorphism (SNP) markers all analyzed in one reaction. This provides an easily accessible way to attain the extra sequence data contained within STR repeats that cannot be extrapolated from the allele size alone.

A series of DNA mixtures containing two, three, and four contributors have been prepared and run in duplicate for both a standard capillary electrophoresis-based genotyping method and with the ForenSeq™ kit utilizing the Illumina® MiSeq® massively parallel sequencing platform. Mixtures have been run with final DNA input amounts of 500pg, 250pg, and 125pg to simulate a range of scenarios that might be typically encountered during forensic casework and explore how the stochastic amplification problems associated with low-level DNA impact the efficacy of mixture analysis.

Data analysis for the ForenSeq™ results was undertaken with the provided Illumina® universal analysis software suite to determine the STR allele repeat sequence, and additionally from the raw data using a bespoke bioinformatics pipeline that allowed further analysis of any SNPs present within the Polymerase Chain Reaction (PCR) flanking regions. Freely available continuous and semi-continuous mixture interpretation models have been used to evaluate the DNA mixtures with respect to a set of suspect/victim reference profiles providing a likelihood ratio relevant to the hypothesis.

This presentation will assess what benefit the knowledge of allele sequence data brings to this mixture interpretation process and how mixture interpretation, especially for complex touch DNA samples, may evolve in the near future.

Mixtures, Massively Parallel Sequencing, Interpretation

B103 The Power of Massively Parallel Sequencing for Complex Mixture Deconvolution and Other Forensic Applications

Sarah Cavanaugh, 10430 Furnace Road, Ste 107, Lorton, VA 22079; Katie Kennedy, BS, The Bode Technology Group, Inc, 10430 Furnace Road, Ste 107, Lorton, VA 22079; Michael N. Parsons, MS, 10430 Furnace Road, Ste 107, Lorton, VA 22079; Andrew B. Feldman, PhD, 11100 Johns Hopkins Road, Laurel, MD 20723-6099; Jeffrey Lin, MS, Johns Hopkins Applied Physics Lab, 11100 Johns Hopkins Road, Laurel, MD 20723; Jeffrey Becker, MS, Johns Hopkins Applied Physics Lab, 11100 Johns Hopkins Road, Laurel, MD 20723; Jon Davoren, MS, 10430 Furance Road, Ste 107, Lorton, VA 22079; and Donia Slack, MS, 10430 Furnace Road, Ste 107, Lorton, VA 22079*

After attending this presentation, attendees will learn of the multiple applications that massively parallel sequencing, or next generation sequencing, has for human identification and forensic investigations.

This presentation will impact the forensic science community by discussing complex mixture deconvolution through the sequencing of the Combined Offender DNA Index System's (CODIS's) core Short Tandem Repeats (STRs). The analysis of Single Nucleotide Polymorphisms (SNPs) for identity, phenotype, and ancestry on compromised or challenging evidentiary items will also be addressed.

With rapidly improving chemistries and decreasing cost, massively parallel sequencing has incredible potential for forensic investigations. Sequencing forensic STRs can overcome some of the limitations of genotyping by capillary electrophoresis and provides increased statistical significance with backward compatibility to size-based methodologies. The information provided by massively parallel sequencing can be invaluable for the deconvolution and analysis of complex DNA mixtures often obtained from forensic evidence, including items handled by multiple contributors and samples taken from rape cases involving multiple suspects. Additionally, this methodology allows for the analysis of large panels of other forensically relevant DNA markers, such as SNPs for identity, phenotype, and ancestry. These markers can be used to provide investigative leads and, due to their small size, can be exploited for use on highly degraded samples, such as aged evidence or skeletal remains.

Bode Cellmark Forensics, Inc. tested forensically relevant samples, such as handled documents, gun grips, tool handles, blood, semen, and saliva utilizing a novel sequencing kit and software solution as well as several commercially available next generation forensic kits; all specifically designed for use on the Illumina® MiSeq® platform. These techniques allowed for the analysis and data interpretation of massively parallel sequencing data of forensic loci of interest, from which the deconvolution of complex DNA mixtures was accomplished through the exploitation of SNPs within and immediately flanking STR loci. Additionally, the sequence variants analyzed provided an advantage over traditional capillary electrophoresis technologies by adding statistical power to match probabilities, forensic likelihood ratios, and paternity indices. In a panel of 92 individuals comprised of Caucasians, African Americans, Han Chinese, and Mexican Americans, D21S11 demonstrated a match probability of 1 in 23. By analyzing the SNP variants within and surrounding this locus, the match probability increased almost three-fold to 1 in 60. Furthermore, these samples were shown to be successfully typed for not only CODIS STR loci, but also SNPs for ancestry, all matching to the self-proclaimed ancestral origins of the donors.

Notably, the majority of samples sequenced were able to be successfully analyzed at the sub-nanogram level, including degraded blood and skeletal remains. The results of this comprehensive evaluation demonstrated the vast utility that massively parallel sequencing has for forensic applications, not only through the analysis of STRs, but also through the analysis of forensically relevant SNP markers for investigative leads.

Next Generation Sequencing, SNPs, Mixtures

B104 Differentiation of Individual Contributors in Contact Epidermal Cell Mixtures Using Fluorescently Labeled Antibody Probes, High Resolution Microscopy, and Flow Cytometry

Cristina E. Stanciu, BS, Virginia Commonwealth University, 1015 Floyd Avenue, Rm 2015, Richmond, VA 23284; Kate Philpott, JD, 9014 Falls Run Road, McLean, VA 22102; Ye Jin Kwon, MS, 640 Worcester Road, #502, Framingham, MA 01702; Eduardo E. Bustamante, BS, Virginia Commonwealth University, 1015 Floyd Avenue, Richmond, VA 23284; Tracey Dawson Cruz, PhD, Virginia Commonwealth University, 1015 Floyd Avenue, PO Box 843079, Richmond, VA 23284; and Christopher J. Ehrhardt, PhD, Virginia Commonwealth University, Dept of Forensic Science, 1015 Floyd Avenue, Rm 2015, Richmond, VA 23284*

After attending this presentation, attendees will understand the biochemical variation within epidermal cells across individuals and how this diversity can be coupled to antibody-based tagging procedures to differentially label contributors in a biological mixture. Attendees will also learn how flow cytometry and Fluorescence Activated Cell Sorting (FACS) may be used to rapidly characterize complex cell populations and physically isolate individual cell populations from a mixture sample.

This presentation will impact the forensic science community by introducing a new strategy for resolving touch mixtures that involves physical separation of cell populations before DNA analysis. This technique can assist forensic laboratories by providing an alternative to complex mixture interpretation procedures, thereby reducing analytical subjectivity and loss of evidence.

Analysis of contact or "touch" mixtures is a significant problem for DNA caseworking laboratories. The presence of cells from multiple contributors in an evidence sample can produce Short Tandem Repeat (STR) profiles that are difficult or impossible to interpret. Although many protocols for cell separation exist, most cannot be applied to mixture samples with only one cell type. New methods are needed that can differentiate epidermal cell populations from different contributors and allow them to be physically isolated prior to DNA profiling. One promising strategy for resolving complex cell mixtures is to target the diversity in protein structures in cells from different individuals. Differentially expressed proteins can be tagged with molecular antibody probes and used to label each contributor's cells in a mixture. Although this strategy is routinely used in biomedical applications, it has rarely been tested in a forensic context. Therefore, the goals of this study were to survey biochemical variation in epidermal cell populations and to identify specific protein targets that may be used to differentially label and sort individual cell populations from a touch mixture.

"Touch" epidermal cells were collected from ~20 individuals and characterized using high-resolution microscopy and flow cytometry. Analysis of unstained samples showed that the touch samples were composed of two distinct fractions: one containing keratinocytes ~30µm-50µm and the other composed of cell fragments/debris <10µm. Keratinocyte populations from each donor were isolated and hybridized to antibody probes targeting the Human Leukocyte Antigen (HLA) complex and cytokeratin filaments within the cell. Hybridization of epidermal cells with allele-specific HLA probes showed no discernable increase in fluorescence compared to unstained controls and subsequent experiments utilizing pan-HLA probes exhibited similar trends. This suggests that, unlike other cells types (e.g., White Blood Cell (WBC) and buccal cell), HLA antigens are either inadequately expressed or insufficiently reactive on epidermal cell surfaces to be a suitable target for cell labeling.

Next, epidermal cells were hybridized with cytokeratin probes AE1 and AE3 which target different sets of filament proteins. Fluorescence levels of AE1-hybridized cell populations showed significant differences across donors with median fluorescence ranging from ~700 to ~11,000 Relative Fluorescence Units (RFUs) and some donors showing as much as a two-fold increase in intensity over other individuals. Hybridization with AE3 probe showed similar trends with median fluorescence of cell population from different donors ranging from ~200 to ~600 RFUs. This indicates that AE1/AE3 antibody probes may be a useful tool for capturing biochemical variation between donor populations and differentially labeling cells in a mixture.

To test whether these biochemical differences could be used to separate cell populations in a forensic sample, touch mixture samples were labeled with AE1 probe and processed using FACS. Isolated cell populations were then subjected to STR profiling. Results showed that touch epidermal cells from different contributors could be labeled and then sorted, intact, with high efficiency into separate reservoirs (~95% of input cells captured). While partial to complete 23-locus STR profiles were obtained from the majority of sorted cell populations, DNA yields from these fractions vis-à-vis cell count suggest that intracellular keratinocyte DNA is highly degraded and that a significant portion of amplifiable DNA from contact epidermal samples may be extracellular. Thus, flow cytometry-based strategies for sorting the most challenging complex mixtures (i.e., mostly epidermal) may need to be coupled to sensitive DNA profiling techniques as well as workflows for processing the extracellular fraction of the mixture sample, which can easily be isolated during FACS for direct amplification.

Mixture Interpretation, STR Profiling, Flow Cytometry

B105 Cell Separation of Multiple Contributor Samples to Facilitate DNA Mixture Analysis

Nancy A. Stokes, MS, Virginia Commonwealth University, 1015 Floyd Avenue, PO Box 843079, Richmond, VA 23284; Cristina E. Stanciu, BS, Virginia Commonwealth University, 1015 Floyd Avenue, Rm 2015, Richmond, VA 23284; Christopher J. Ehrhardt, PhD, Virginia Commonwealth University, Dept of Forensic Science, 1015 Floyd Avenue, Rm 2015, Richmond, VA 23284; and Susan Greenspoon, PhD, Department of Forensic Science, 700 N 5th Street, Richmond, VA 23219*

After attending this presentation, attendees will better understand a possible new solution for simplifying Short Tandem Repeat (STR) genotyping of samples derived from multiple contributors. This technique involves separation of cells by the process of Fluorescence Activated Cell Sorting (FACS) prior to DNA extraction and amplification, thus enriching for contributors the resulting fractions and enhancing statistical strength of the genotyping analysis.

This presentation will impact the forensic science community by illustrating that forensic DNA evidence samples comprised of multiple individuals can significantly complicate interpretation of the resulting STR profiles and decrease the strength of the statistical analyses. In order to separate DNA sources within a mixture sample prior to STR analysis, FACS was utilized to segregate donor cells, based upon cellular characteristics of the contributors within the sample. The resulting separate fractions have reduced complexity, thereby facilitating STR analysis of the mixture sample.

FACS was utilized to separate contributor cells within Human Leukocyte Antigen (HLA) -labeled blood mock evidence mixture samples and mock touch evidence mixture samples based upon cell morphology. DNA was obtained from the mixture (presorted) and FACS sorted samples by the DNA IQ™ system. STR amplification was performed with the PowerPlex® Fusion System and resulting profiles were compared for the presorted and sorted samples. Genotyping was conducted manually for all profiles and by the probabilistic modeling program TrueAllele® Casework (TA) for selected profiles.

Presorted blood mock evidence mixture samples from three and four contributors yielded complex STR profiles. One of the sorted blood fractions from both three and four contributor mixtures showed enrichment to the level of one or two individuals. The other fraction was still a mixture but was less complex (i.e., at least one contributor was almost completely selected out). TA analysis of these presorted and sorted samples demonstrated enhanced statistical power post-sorting. Presorted samples for mixtures of individuals 105, 106, and 107 had similar log Likelihood Ratio (log(LR)) values for each contributor (9.86, 11.40, and 9.65, respectively). For the two FACS-sorted cell populations, TA analysis of fraction P2 produced a slightly higher value for contributor 106 (12.34) and excluded 105 and 107 (negative log(LRs) generated). TA analysis of fraction P3 produced a slightly higher value for 105 and nearly 11 orders of magnitude higher value for 107, respectively (10.13, 20.56) and excluded 106. Moreover, TA analysis of the sorted mixture from four individuals produced a log(LR) value to approximately single-source level (28.18) for contributor 103 in fraction P2. TA analyses thus confirmed and quantified observations by analyst genotyping that enrichment for individual contributors and increased statistical power may be accomplished with the use of FACS for cell sorting.

Mock epithelial touch evidence samples were analyzed as to whether the source of DNA was extracellular (free DNA), intracellular, or both. Intracellular DNA was isolated from pelleted and washed presorted cells and free DNA was collected from the supernatant of the washed cells and subjected to Microcon-100 concentration. Sorted cells yielded only intracellular DNA as the washes and sorting process removed free DNA from the sample. Polymerase Chain Reaction (PCR) quantification of presorted DNA showed that intracellular DNA was present, but the ratio of free DNA to intracellular DNA was approximately 2:1, though genotyping data were generated from both DNA sources. Epithelial samples were FACS sorted based on cell morphology (>20µm or <20µm). Six epithelial touch mock evidence mixture samples, each comprised of two or three contributors, were FACS sorted. The >20µm fraction yielded partial profiles for a single donor from two samples, inconclusive results from two samples, and no results from two samples. The free DNA yielded a full STR profile for a single donor from one of the samples, a partial single-source profile from four samples, and one sample failed to yield any results. These results demonstrate that FACS separation may be performed on touch evidence based upon cell morphology after additional protocol development and that at least a portion of the DNA from touch evidence is intracellular in origin.

Cell Sorting, Mixture Samples, Probabilistic Genotyping

B106 Separation of Compromised Blood Mixtures Using Fluorescence-Activated Cell Sorting (FACS) for Single-Source Short Tandem Repeat (STR) Profiling

Cristina E. Stanciu, BS, Virginia Commonwealth University, 1015 Floyd Avenue, Rm 2015, Richmond, VA 23284; Ye Jin Kwon, MS, 640 Worcester Road, #502, Framingham, MA 01702; Sarah R. Ingram, BS, Virginia Commonwealth University, 1015 Floyd Avenue, Rm 2015, Richmond, VA 23284; and Christopher J. Ehrhardt, PhD, Virginia Commonwealth University, Dept of Forensic Science, 1015 Floyd Avenue, Rm 2015, Richmond, VA 23284*

After attending this presentation, attendees will better understand how FACS coupled with Human Leukocyte Antigen (HLA) labeling may be used to resolve complex blood mixtures that have been degraded or compromised.

This presentation will impact the forensic science community by introducing a new method for separating contributors prior to DNA extraction, which can improve the efficacy of mixture interpretation in DNA caseworking units.

Previous studies have demonstrated that hybridizing cell mixtures with fluorescently labeled antibody probes and FACS can be an effective technique for separating certain types of cell mixtures. These include sperm and epithelial cell mixtures, where each cell can be easily differentiated based on their biochemical composition and morphological properties. Mixtures containing only one cell type have proven more difficult to resolve using this approach, especially when the mixture has been compromised and/or degraded. Sorting the cells from such samples has proven to be a challenge due to the complex chemical and physical changes associated with cellular decomposition, which lead to non-specific fluorescence and significant sample loss. The objective of this study was to develop new methods for labeling and sorting cells in compromised mixture samples and subsequently test them against samples approximating those encountered in forensic casework.

To accomplish this, a series of two- and three-person whole blood mixtures was created in which the total mixture volume ranged from 100µl-500µl, and each contributor was present in equal volume ratio. Mixtures were dried for 12 hours and then hybridized to antibody probes that targeted the HLA-A*02 allele. While flow cytometry analysis showed that dried samples experienced some cell loss, intact, immunoreactive white blood cells could be recovered from all samples (~300-13,000 cells). More importantly, when comparing flow cytometry results of one contributor's dry blood sample against their fresh whole blood sample, the dried blood sample showed similar binding specificity to the HLA-A*02 probe, such that A*02 positive donor(s) could still be resolved from negative donor(s) in mixture samples. Differences in the fluorescence intensity of HLA positive and negative contributor cell populations were subsequently used to define sorting criteria for isolating each mixture fraction. STR profiling was then performed to determine the sorting efficiency and assess the utility of this technique for forensic casework.

STR results from seven different mixtures showed successful isolation of both HLA-A*02 positive and negative contributors from each mixture. Profiles from the positive contributors were identical to their presorted, single-source profiles across 16 different loci. Allelic contributions from non-target contributor(s) was rarely observed, and, when present, was easily resolved from contributor alleles (10:1 to 50:1 contributor to non-contributor ratio). The sorted cell fraction from the HLA negative contributor showed similar results with STR profiles showing at least a ten-fold enrichment in contributor-specific alleles compared to the presorted mixture profile. Overall, these results suggest that HLA antibodies can be used to differentially label cell populations in a compromised blood mixture and, when coupled to FACS, cells from different contributors can be physically separated from a mixture to generate single-source STR profiles.

FACS, Human Leukocyte Antigen, Mixture Interpretation

B107 Development of a New DNA Screening System of Criminal Samples Using ForensicGEM™ and Adhesive Sheets

Shinichiro Akase, PhD, Forensic Science Laboratory, Kagoshima Pref.Police, Kamoike shin-machi 10-1, Kagoshima City, Kagoshima Prefecture 890-8566, JAPAN; Gregory S. Hummel, MS, Kansas City Police Crime Lab, 6633 Troost, Kansas City, MO 64131; Yasuhide Iwata, Forensic Science Laboratory Saga Pref.Police, Matsubara 1 Choume 1-1, Saga City 840-8540, JAPAN; Yuki Kariya, MS, Forensic Science Lab Kagoshima Pref.Police, 10-1 Kamoike shin-machi, Kagoshima City 890-8566, JAPAN; Takeshi Yoshikawa, Faculty of Fisheries, Kagoshima University, 4-50-20 Shimoarata, Kagoshima 890-0056, JAPAN; and Kazumasa Sekiguchi, PhD, 6-3-1 Kashiwanoha, Kashiwa 277-0882, JAPAN*

After attending this presentation, attendees will understand the concept of obtaining a simple idea from the everyday work of a criminalist and the importance of providing inter-agency training.

This presentation will impact the forensic science community by showing the practical possibilities of a new screening system.

For many years, numerous Short Tandem Repeat (STR) analyses have been performed on crime scene biological samples such as blood, saliva, and human body tissue. The information obtained from these analyses is utilized as evidence during criminal investigations. The high number of required analyses causes problems in terms of cost and labor for almost all crime laboratories. One reason for this is that it is impossible to know which analysis will be useful beforehand. In other words, many wasteful analyses are performed to obtain useful results. In a case with numerous blood stains from the offender and the victim, such as murder and injury, it is difficult to find the offender's blood without consuming a large amount of STR reagents and labor as there may also be several victims' stains, mixed stains, and degraded stains at the scene. If the positions of these different types of stains could be known before STR analysis, the number of target stains requiring analysis could be narrowed down. In order to simultaneously extract DNA from many samples present on crime scene material, such as a T-shirt, a new system was designed using a DNA extraction reagent (ForensicGEM™) and an adhesive sheet (polyvinyl chloride).

The essence of this system is as follows: (1) many samples are simultaneously collected on the adhesive sheet; (2) multiple DNA extractions are performed directly on the sheet by ForensicGEM™; (3) multiple DNA extracts are simultaneously subjected to quantitive Polymerase Chain Reaction (PCR); and, (4) the results of quantitive PCR are simultaneously indicated by computer graphic software.

In this study, basic experiments were performed to evaluate the efficiency and accuracy of this procedure for blood stains, saliva stains, and human body tissue samples. In conclusion, sufficient DNA can be extracted from blood and saliva in this manner, but body tissues were less effective. Both blind and situational tests were conducted with hypothetical samples. The results of these tests suggest the possibility of practical use of forensic DNA analysis in the field.

This presentation will also show the application of a bio-robot for attempting to move the solutions from the adhesive sheet to a 96-well plate automatically.

ForensicGEM™, Adhesive Sheet, DNA Screening

B108 Optimized Methods for Collection and Extraction of DNA From Archived Latent Fingerprints

April D. Solomon, BS, Virginia Commonwealth University, 4830 Valley Crest Drive, #202, Midlothian, VA 23112; Madison Hytinen, Virginia Commonwealth University, Richmond, VA 23220; Aryn M. McClain, BS, Virginia Commonwealth University, 1015 Floyd Avenue, PO Box 843079, Richmond, VA 23284; Marilyn T. Miller, EdD, VA Commonwealth University, 1015 Floyd Avenue, Rm 3001A, Box 843079, Richmond, VA 23284-3079; and Tracey Dawson Cruz, PhD, Virginia Commonwealth University, 1015 Floyd Avenue, PO Box 843079, Richmond, VA 23284*

After attending this presentation, attendees will understand the link between crime scene collection, visualization methods for latent fingerprints, and the success of laboratory analysis. The objective is to identify the best combination of DNA collection, extraction, and typing methods for treated and untreated latent fingerprints that were tape-lifted, secured on paper backing cards, and stored at room temperature for varying time periods.

This presentation will impact the forensic science community by providing more efficient collection and DNA extraction pairings, which are ready for immediate implementation, and by improving the success rate for obtaining probative Short Tandem Repeat (STR) profiles from tape-lifted archived fingerprints. Currently, the forensic DNA literature has not addressed this specific type of challenged sample.

Touch DNA samples have grown since the late 1990s due to advanced forensic technology, giving the potential to provide probative evidence for criminal casework, even in the absence of body fluids. Several studies have evaluated the best collection methods for latent fingerprints when DNA profiling is anticipated; others have separately reported DNA success from adhesives and from paper material. However, there is limited information on best practices for retrieving DNA from latent fingerprints sandwiched between adhesive and paper substrates.[1] Since these samples are often stored for long time periods, likely contain low template DNA, and end in failed analysis or lead to irresolvable mixtures, they are often overlooked as viable DNA evidence. Unfortunately, for many older cases, archived latent fingerprints collected in this manner may be the only physical or biological evidence available. With touch DNA consisting of corneocytes (dead skin cells) mixed with sweat and body oil, the potential for successful typing is high for these archived samples; however, data on success rates, optimized methods, and mixture prevalence is needed before laboratories agree to routinely process these samples.[2,3]

In this study, ten participants provided a set of latent fingerprints on both non-porous (glass) and porous (paper) surfaces. Fingerprints were treated with traditional powders for visualization, tape-lifted, and secured on paper substrates. Initially, single fingerprint cuttings were obtained and DNA recovery was assessed using four extraction methods (phenol-chloroform organic extraction, Qiagen QIAamp® Investigator kit, Invisorb® Spin Forensic kit, and ZyGEM® prepGEM™ Tissue kit). These findings confirmed that human cells (and thus, DNA) are retained on both the adhesive and paper sides of archived fingerprints. The paper substrate held more than three times as much DNA as the adhesive side. Thus, both the paper and adhesive sides should be processed in a combined procedure to improve DNA yields. Overall, the Investigator kit provided more detectable DNA than the other methods (53% of all samples tested). Respectively, DNA from the Invisorb® kit, ZyGEM® kit, and organic extraction provided detectable DNA from 43%, 23%, and 10% of all samples tested. Of the samples detected during quantitation, those processed with the Investigator kit provided the most consistent total DNA yields (1.643ng average); however, when visualization techniques were considered, results varied among the fingerprint treatments: magnetic treated, black carbon powder treated, and untreated fingerprints provided the highest DNA yield with the Investigator kit (2.00446ng average), organic extraction (0.6024ng average), and ZyGEM® kit (0.68322ng average), respectively. Thus, the visualizing technique performed at the crime scene may dictate which extraction will perform most efficiently when preparing archived fingerprints for DNA analysis. Interestingly, fingerprints processed using magnetic powder or black powder produced more detected samples and more DNA (on average) than untreated fingerprints. Based on this data, the Investigator kit was employed to evaluate the best methods for actually retrieving DNA from the paper and adhesive substrates of archived samples. Cuttings, a single-swab technique, and a double-swab technique were compared using multiple swab diluents (distilled water and 2% sodium dodecyl sulfate). Thus far, the double-swab technique is providing more than three times the DNA on average (0.497ng) than the single-swab technique (0.108ng) using water as the diluent.

In conclusion, this experiment will provide valuable information for forensic DNA examiners seeking to process archived fingerprints for DNA typing. The recommendations will be readily available for immediate execution to increase the likelihood of obtaining a full STR profile for casework. As such, cold cases with limited physical and biological evidence could be revisited with more confidence and less apprehension about these potentially compromised, low template DNA samples.

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Touch DNA, Archived Fingerprints, DNA Typing

B109 Comparison of DNA Yield and Short Tandem Repeat (STR) Success Rates From Various Tissues in Embalmed Bodies

Amanda Wheeler, BS, 1105 Beasley Hills Lane, Houston, TX 77008; Natalia Czado, MS, 43 Virginia Avenue, Woonsocket, RI 02895; David A. Gangitano, PhD, Sam Houston State University, 13906 Paradise Valley Drive, Houston, TX 77069; and Sheree R. Hughes-Stamm, PhD, Sam Houston State University, Dept of Forensic Science, Huntsville, TX 77341*

After attending this presentation, attendees will understand some of the principles of genotyping chemically damaged and degraded tissue samples, such as those harvested from embalmed cadavers, for identification.

This presentation will impact the forensic science community by providing guidance concerning which tissues may be best harvested from embalmed bodies and those that will provide the highest quantity and quality DNA for human identification purposes in a timelier manner.

The process of formalin fixation is commonly used to preserve tissue sections for pathological testing and embalming cadavers for medical use or in preparation for burial. DNA extracted from formalin-fixed tissues may provide an alternative source of material for identification in forensic cases. Formaldehyde causes DNA damage and degradation, thereby reducing the quantity and quality of DNA available for downstream genetic analysis.

By analyzing the DNA yield, level of DNA degradation, and STR success of various tissues from embalmed cadavers, some guidance may be provided to forensic analysts regarding which samples from embalmed bodies are likely to generate more complete STR profiles.

In this study, tissue samples (N=122) were dissected from three male embalmed cadavers and included bone, cartilage, hair, muscle, internal organs, skin, teeth, and nail clippings. DNA was purified from all samples, the DNA quantity and level of degradation was determined using the QuantiFiler® Trio DNA Quantification kit, and genotyped using the GlobalFiler® Polymerase Chain Reaction (PCR) Amplification kit.

The results of this study, which showed a wide variation in DNA yield, degradation, and STR success between different types of tissues within each cadaver, and some variation between the three cadavers, will be presented. Overall, bone marrow samples resulted in the highest DNA yields, lowest DNA degradation values, and highest STR success; however, various muscle and skin samples also provided complete STR profiles, demonstrating that some soft tissues that are more quickly and easily harvested from embalmed cadavers can provide the same or greater DNA yields and STR success than the traditional, and more difficult to collect and process, bone and tooth samples.

When comparing tissues that experienced blood pooling (due to livor mortis) to tissues in regions that experienced compression, significantly more complete STR profiles on average were generated from the compressed tissues (62% versus 37% alleles recovered) (Analysis of Variance (ANOVA); p=0.02). These data support the thought that tissues affected by lividity may also experience greater exposure to formalin, resulting in DNA damage and reduction in downstream STR success.

Embalmed, DNA Damage, Tissue Sampling

B110 Standardized Kinship Data Test Set for Rapid DNA Validation

Stephanie DeDore, BS, 5200 S Merrimac Avenue, Chicago, IL 60638; Yvette Crandall, MS, BAE Systems, Inc, 16541 Commerce Drive, King George, VA 22485; Daniele S. Podini, PhD, Department of Forensic Science, 2100 Foxhall Road, NW, Washington, DC 20007; and Amanda C. Sozer, PhD, 4707B Eisenhower Avenue, Alexandria, VA 22304*

After attending this presentation, attendees will understand how the development of a standardized kinship data test set can be a simple and effective tool to aid in the validation of Rapid DNA kinship software. Attendees will also learn the planning and design strategies required for developing such a test set, enabling them to develop a test set for their own validation needs.

This presentation will impact the forensic science community by serving as a tool for a quick, simple, and effective method to validate the kinship software associated with Rapid DNA technology in preparation for field or laboratory DNA analysis operations. This validation tool will create standardization of the Rapid DNA platform's kinship functionality across all laboratories and fieldwork locations.

The Department of Homeland Security's (DHS's) mission is to protect America by preventing terrorism and enhancing security, managing the borders, administering immigration laws, securing cyberspace, and ensuring disaster resilience. While DNA is a powerful biometric, currently DHS does not have in-house DNA testing capabilities. Rapid DNA, a recent and important technology advancement in DNA testing, is designed to be utilized as a lights-out system with the ability to provide results within 90 minutes. This technology provides DHS with a portable, robust, in-house DNA capability. Rapid DNA technology for DHS kinship analysis was developed through a DHS Science and Technology Directorate (S&T) research and development effort that was part of a collaborative effort with the Departments of Defense and Justice. Through the use of Rapid DNA technology, DHS components may be able to prevent illegal and fraudulent immigration, deter human trafficking, identify disaster victims and reunite families, and identify criminals or terrorists attempting to enter the country. For this reason, the Rapid DNA kinship determination functionality will be of particular use to DHS.

The Rapid DNA kinship functionality will require validation and verification before the technology is put into operation and occasionally when the Rapid DNA software is updated or modified. In order to make the Rapid DNA kinship software validation and verification universal across all components of DHS, a robust kinship data test set has been created to enable quick and simple validation. When the Rapid DNA system is updated, the user can simply run the kinship data test set to ensure no errors or issues have occurred in the kinship analysis and statistical calculation process. When a kinship test is run on Rapid DNA, the results will show either inclusion or exclusion and the calculated relationship indices. The Rapid DNA user will be able to detect potential changes applied to the Rapid DNA kinship application and determine if the statistical analysis for the DNA profiles are reported properly.

Expanding on the National Institute of Standards and Technology's Standard Reference Family Data approach (http://www.cstl.nist.gov/strbase/kinship.htm), Common Message Format (CMF) files containing profiles associated with ten artificial pedigrees have been created. These pedigrees are specifically designed to include a wide number of alleles, including off-ladder alleles and microvariants. The kinship cases include biological relationship/kinship claims commonly encountered by the United States Citizenship and Immigration Services (USCIS) and Customs and Border Protection (CBP): standard mother/father/child trios, motherless, fatherless, grandparent-grandchild, siblings, and aunt/uncle to niece/nephew cases. The cases also include other DNA profile complexities (e.g., parent-wrong gender, mutations, null alleles, and rare alleles). The kinship data test sets incorporate the 20 common paternity index formulas identified by the American Association of Blood Banks (AABB). Testing of paternity index formulas for paternity trios and motherless cases are in accordance with the 2014 Appendix 4 of the AABB *Guidance for Standards for Relationship Testing Laboratories* 11th Edition.

Overall, this kinship data test set will provide not only DHS but all Rapid DNA users with a standardized validation tool allowing for easy implementation and validation of Rapid DNA technology as well as providing a resource for users to create their own kinship test set.

Rapid DNA, Validation, Kinship

B111 Development of a Microfluidic Differential Extraction Module and Refinement of Infrared (IR) -Mediated Short Tandem Repeat (STR) Amplification for a Rotation-Driven Microdevice

Kemper Gibson, 2230 E Teemont Court, Richmond, VA 23225; Jordan Cox, MS, 1823 Floyd Avenue, Richmond, VA 23220; Kimberly Jackson, 409 McCormick Road, Chemistry Dept, Charlottesville, VA 22903; James P. Landers, PhD, University of Virginia, Dept of Chemistry, McCormick Road, Charlottesville, VA 22904; and Tracey Dawson Cruz, PhD, Virginia Commonwealth University, 1015 Floyd Avenue, PO Box 843079, Richmond, VA 23284*

After attending this presentation, attendees will better understand the forensic applications of microfluidic platforms, micro Total Analysis Systems (μTAS), and recent advancements toward integrating a differential DNA extraction module for sexual assault samples.

This presentation will impact the forensic science community by describing a differential extraction module on a rotation-driven microdevice platform for sexual assault casework samples as well as refinement of IR-mediated STR amplification occurring on the device. If implemented, an automated device based on this microchip could remove the human variability often seen with manual differential extractions, speed the workflow for sexual assault samples, and result in high-quality data with fewer STR mixtures needing lengthy interpretation.

Microdevices offer several potential advantages over conventional methods for quick determination of STR profiles, including smaller reagent and sample volumes, less manual intervention, and decreased risk of contamination — all leading to decreased sample-to-answer time. IR-Polymerase Chain Reaction (PCR) and enzyme-driven DNA preparation have improved the ability of scientists to integrate microdevice modules that represent all aspects of the traditional forensic DNA workflow. While these technologies have the potential to significantly impact the way forensic science DNA laboratories function, extensive modification of these technologies is needed before microdevices can be implemented for processing casework samples. Commercially-available microdevice-based instruments (i.e., Remote Access Program for Interactive Diagnostics (RAPID)) require extensive external pumps and actuators for microfluidic control and are designed only for the processing of single-source reference (known) buccal swab samples. Unfortunately, forensic casework samples, ~52% of which involve testing of sexual assault evidence and which require a differential cell lysis process, are not currently amenable to microchip-based processing. Manual differential lysis is a time-consuming DNA purification process that often still results in mixtures, which require a significant amount of interpretation effort. As the media draws more attention to the number of rape kits left untested each year, it will become imperative that crime laboratories find ways to process more of these samples more quickly. To this end, the first goal of this project was the incorporation of a differential extraction module onto the aforementioned microdevice.

In this project, sperm cells were separated from non-sperm cells via an antibody-labeled, bead-based capture mechanism. Initially, chip architecture was redesigned to include an antibody capture chamber that allows for dual valving and microfluidic movement into side-by-side sperm cell and non-sperm cell DNA liberation chambers. This design allows for antibody-coated microbeads to selectively capture sperm cells, which would prevent movement through a burst valve designed to capture non-sperm cells. Next, two sperm-specific antibodies (SP-10 and SPAG8) and one male-specific antibody (MEA-1) were tested off-chip for binding efficacy using flow cytometry. To assure that other contributing female and epithelial cells were not binding to the sperm antibodies, two antibodies specific for epithelial cells were also used. Based on binding specificity and efficiency results from the flow cytometry data, the best sperm-specific antibody was selected for further on-chip testing.

The second objective of this work was to explore STR reaction chemistry alterations in an effort to improve STR profile issues often seen with IR-mediated amplification. Existing microdevice platforms that utilize rapid small volume IR-PCR and polymerase combinations often lead to the presence of significant non-adenylated (-A) products and an overall "ski-slope" effect that leaves alleles in the larger-sized loci either very low or undetectable by capillary electrophoresis. In previous studies, a commercially available primer set was used along with Phusion® Flash master mix and SpeedSTAR™ HS polymerases in a reduced volume reaction for IR-mediated on-chip amplification. In this study, AmpliTaq Gold® polymerase and AmpFℓSTR® Identifiler® Plus chemistry were evaluated, along with adjustments to the original combination of Phusion® Flash and SpeedSTAR™ HS, other enzyme combinations (AmpliTaq Gold® Fast and KAPA2G Fast), and longer final extension times. When used alone, AmpliTaq Gold® yielded negative Identifiler® Plus results; however, using it in combination with the Phusion® Flash-SpeedSTAR™ HS combination and a longer final extension (180s) resulted in a nearly complete profile (29 of 30 alleles called) with more than 40% of alleles (12 of 29) showing greater adenylated product. Additionally, none of the STR loci showed *only* -A peaks. Taken together, these improvements represent large strides toward the development of a sexual assault microdevice.

Microdevice, Differential Extraction, IR-PCR

B112 Direct Amplification and Commonly Encountered Crime Scene Substrates

Katelyn M. Gigl, BS, Penn State University, 107 Whitmore Lab, University Park, PA 16802; and Reena Roy, PhD, Pennsylvania State University, Forensic Science Program, 325 Whitmore Lab, University Park, PA 16802*

After attending this presentation, attendees will better understand how to obtain DNA profiles by direct amplification of bloodstains on substrates commonly encountered at crime scenes. Attendees will also learn that it is possible to obtain complete DNA profiles from body fluids without extraction or pretreatment of the stains.

This presentation will impact the forensic science community by providing information concerning how to generate DNA profiles within a very short time and without labor-intensive and time-consuming steps.

Items of evidence retrieved from crime scenes often contain blood. In order to generate Short Tandem Repeat (STR) profiles from such evidence, extraction, purification, and quantification prior to amplification of DNA is necessary. In this research, autosomal STR profiles were generated via direct amplification of bloodstains deposited on simulated crime scene substrates.

Blood samples from deceased males were collected in sterile tubes containing Ethylenediaminetetraacetic Acid (EDTA) to prevent DNA degradation and were kept frozen until ready for analysis. There were four stages in this study. The blood samples from the deceased donors were first extracted using the BioRobot EZ1 workstation and EZ1 DNA investigator kit in order to generate reference profiles. In the second stage, a measured volume (0.1µL) of blood was deposited on each of the ten substrates and dried for 24 hours at room temperature. The objects included: cigarette butt, drinking straw, dry brown leaf, woodchip, leather, and five different types of fabrics often encountered as evidence from crime scenes. None of these objects contained any lysing agent. Several of these objects contained potential inhibitors. The deposited bloodstains were punched using a Harris 1.2mm micro-punch on nine of the listed substrates. The tenth substrate, the woodchip, was too difficult to punch. Therefore, a minute piece approximating the 1.2mm punch was shaved off after depositing the blood on the woodchip. Each of the stains was extracted using the forensicGEM™ tissue extraction protocol. The samples generated from the two extraction methods were quantified and amplified using the amplification kits used for direct amplification.

In the next step of the project, each of these punched substrates containing the bloodstains created from 0.1µL of blood was amplified directly after pretreatment with reagents and buffer. All the substrates containing the stains were treated with the Prep-n-Go™ buffer prior to amplification with the GlobalFiler® Express Amplification kit. SwabSolution™ and PunchSolution™ were used for treatment of the stains prior to amplification with the PowerPlex® Fusion and PowerPlex® 18D Systems.

In the fourth and final stage of the research, each stain was amplified directly without any pretreatment with the reagents and buffer mentioned earlier. During the third and the fourth stages of this research, the substrates remained in the amplification reagents during the thermal cycling steps. Each bloodstain was amplified with the three direct autosomal STR amplification kits: GlobalFiler® Express Amplification kit, PowerPlex® Fusion, and PowerPlex® 18D Systems. Capillary electrophoresis was performed on a 3130xl Genetic Analyzer and data were analyzed using **GeneMarker**® software version 2.7.1.

Reference profiles were obtained from blood extracted using the traditional (EZ1 extraction) method. These profiles were compared to the profiles generated from the stains deposited on the various substrates and extracted with the forensicGEM™ tissue extraction protocol. The profiles were found to be concordant and consistent between and within each substrate and each amplification kit. These profiles were then compared to the profiles generated from each of the stains amplified directly with and without pretreatment as described in the third and fourth stages of the research.

Complete and concordant autosomal STR profiles were successfully obtained from the bloodstains deposited on the ten challenging substrates when they were amplified directly using GlobalFiler® Express Amplification kit, the PowerPlex® Fusion, and the PowerPlex® 18D Systems. The research indicated that the pretreatment of the bloodstains with the reagents and buffer did not enhance the quality of the profiles. Complete and concordant profiles were obtained when the stains were not subjected to any pretreatment and while the substrates remained in the reagents during the amplification steps.

At this time, the forensic community requires that forensic DNA analysts perform quantification prior to amplification. This study with direct amplification using simulated casework-type samples may be of limited value. Currently, with instruments such as the RapidHIT™ system from IntegenX® or the DNAScan Rapid DNA Analysis System from NetBio, analysts are already performing direct amplification of body fluids. It is expected that this procedure, which involves challenged substrates and direct amplification, would be acceptable in the near future.

Direct Amplification, STR, Crime Scene

B113 Tissue Preservation With Direct-to-Polymerase Chain Reaction (PCR) for DNA Profiling: An Alternative Disaster Victim Identification (DVI) Approach

Amy E. Sorensen, MSFS, 11 Webb Creek Place, The Woodlands, TX 77382; Clare Berry, BAS, National Centre for Forensic Studies, University of Canberra, Canberra, AUSTRALIA; David Bruce, PhD, NSW Forensic and Analytical Science Service, Weerona Road, Sydney, AUSTRALIA; Michelle Gahan, PhD, National Centre for Forensic Studies, University of Canberra, Canberra, AUSTRALIA; Sheree R. Hughes-Stamm, PhD, Sam Houston State University, Dept of Forensic Science, Huntsville, TX 77341; and Dennis McNevin, PhD, National Centre for Forensic Studies, University of Canberra, Canberra, AUSTRALIA*

After attending this presentation, attendees will be informed regarding the efficiency of various solutions to preserve DNA in fresh and decomposing tissue samples when stored in warm ambient temperatures for up to four weeks. In addition to the benefits of storing samples at room temperature, this presentation will also describe the results of combining these preservatives with direct-to-PCR amplification in order to process a high volume of tissue samples for faster DNA identification.

This presentation will impact the forensic science community by addressing the demands for DNA preservation in rapidly decomposing remains and providing faster DNA identification during a mass disaster. By directly amplifying DNA in solution (with dilution in some cases), DNA extraction from the dense tissues can be avoided and successful Short Tandem Repeat (STR) profiles can be obtained in a timelier manner.

Tissue preservation offers the ability to stabilize and isolate DNA from tissues in the field, far from a laboratory setting, where refrigeration may not be available. This has potential application to Disaster Victim Identification (DVI) as well as to any form of field-based forensic biological evidence or intelligence collection.[1] Forensic DNA analysis is one of the three primary methods of identification recommended by the International Criminal Police Organization (INTERPOL), together with fingerprint and dental analysis.[2]

Previous work has demonstrated the ability to obtain full STR profiles from DNA extracted from fresh muscle tissue preserved in Tris, EDTA, NaCl, Tween 20 (TENT) buffer, salt-saturated DMSO-EDTA solution (DESS), and two proprietary preservatives: DNAgard® from Biomatrica® and one from DNA Genotek, Inc.[3] Three of the preservatives (DESS, DNAgard®, and DNA Genotek) also yielded full profiles from DNA extracted from aliquots of the preservative solution surrounding the muscle tissues. Therefore, in this study, the possibility of obtaining DNA profiles without DNA extraction, by adding aliquots of preservative solutions surrounding fresh and decomposing human tissue samples directly to PCR, was explored.

The results of this work, in which full PowerPlex® 21 and GlobalFiler® STR profiles were obtained from fresh and decomposed tissue preserved at 35oC for up to 28 days, as well as from fresh tissue which had been stored at 35oC for up to 28 days, and then at -80oC for four years, will be presented.

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DNA Preservation, Direct-to-PCR, DVI

B114 Enhanced DNA Extraction Via the Reduction and Alkylation of Disulfide Bonds by Iodoacetamide (IAM) and Tris(2-carboxyethyl)phosphine (TCEP)

Megan E. Grimes, MFS, 5187 Salt Pond Place, Woodbridge, VA 22193; Leah E. Willis, PhD, 2501 Investigation Parkway, Quantico, VA 22135; Jodi A. Irwin, PhD, 2501 Investigation Parkway, Quantico, VA 22135; Tamyra Moretti, PhD, Nuclear DNA Unit, FBI Laboratory, 2501 Investigation Parkway, Quantico, VA 22135; and Mark F. Kavlick, BS, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, attendees will understand the importance of reducing and alkylating reagents during forensic DNA extraction, how the selection of these agents can increase DNA yield, and how to incorporate them into a typical casework workflow.

This presentation will impact the forensic science community by providing methods to significantly increase DNA yield from various forensic specimen types (i.e., blood, hair, semen, and calcified tissue) through the use of novel reducing and alkylating reagents which facilitate the breakage of and/or prevention of disulfide bond reformation in cellular proteins during DNA extraction.

Dithiothreitol (DTT) is a key reducing agent used in forensic DNA extraction that facilitates the isolation and purification of DNA from proteins in a biological specimen. DTT reduces protein disulfide bonds to thiols, thereby facilitating protein digestion by proteinase K and permitting the release of DNA from protective and/or contaminating proteins. For example, the unique membrane of spermatozoa, which is rich in disulfide bonds, requires a particularly strong reducing agent to lyse these cells and extract their DNA. Reducing agents are also employed when extracting DNA from hair shafts which are largely composed of keratin, a structural protein which contains myriad disulfide bonds.[1-3]

When using a reducing agent such as DTT, which produces a disulfide exchange, disulfide bonds may reform and may, in turn, reduce DNA yield and/or purity. To prevent such reformation, an alkylating agent, IAM, may be employed in conjunction with DTT. IAM works as an irreversible alkylator by binding to the thiol group of the cysteine residues, thereby preventing the reduced disulfide from reforming. Alternatively, TCEP may be used in lieu of DTT, since it functions as both a reducing agent and an alkylating agent. TCEP also provides many advantages over DTT, such as being odorless and more stable at room temperature.[3,4]

This study examined the effect of IAM and TCEP on the quantity and quality of DNA extracted from hair, calcified tissue, semen, and blood. The following conditions were compared: (1) DTT alone (Standard Operating Protocol (SOP)); (2) DTT followed by IAM alkylation; (3) TCEP; and, (4) TCEP followed by IAM. DNA yields were assessed using nuclear and mitochondrial DNA (mtDNA) -specific quantitative Polymerase Chain Reaction (qPCR) methods and DNA quality was assessed following 16-locus Short Tandem Repeat (STR) analysis.

Both IAM and TCEP are water-soluble and were easily incorporated into a semi-automated SOP. Briefly, the SOP entails lysis of a specimen in buffer G2 from QIAGEN®, proteinase K, and DTT followed by purification on QIAGEN's® EZ1 platform. TCEP treatment simply involved replacement of DTT during the lysis step, at a concentration of 30mM with no further change in the lysis conditions. IAM treatment involved a separate incubation step with 150mM at 22°C for 30 minutes in the dark following the lysis step which included *either* DTT *or* TCEP.

The results revealed that for blood, TCEP treatment significantly increased DNA yield over the SOP by an average of 270%, whereas for semen, TCEP/IAM treatment increased yield over the SOP by an average of 350%. The results also confirmed that TCEP and IAM did not interfere with downstream STR analysis. The benefits of IAM and TCEP were similarly observed for calcified tissue and shed hair. Optimization experiments revealed that TCEP was optimal at 30mM.

In conclusion, the use of IAM, an alternative reducing agent, and TCEP, a reducing/alkylating agent, enhances DNA recovery from hair, calcified tissue, semen, and blood, thus promoting their successful forensic analyses. These enhancements should be particularly valuable for those challenging specimens which contain low copy DNA and/or degraded DNA.

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DNA Extraction, Tris(2-carboxyethyl)phosphine, Iodoacetamide (IAM)

B115 The New Kit on the Block: Optimization of the QIAGEN® Investigator® 24plex GO! Kit for Direct Amplification

Daniel Watsula, MS, 10430 Furnace Road, Ste 107, Lorton, VA 22079; Jon Davoren, MS, 10430 Furance Road, Ste 107, Lorton, VA 22079; and Jangbir Sangha, MA, 10430 Furnace Road, Ste 107, Lorton, VA 22079*

After attending this presentation, attendees will understand how to achieve a high first-pass success rate when performing direct amplification of 1.2mm punches from buccal samples collected on non-treated paper with the QIAGEN® Investigator® 24plex GO! Kit. Attendees will also understand that a high first-pass success rate is achievable with reduced reaction volumes in addition to the standard manufacturer's protocol.

This presentation will impact the forensic science community by demonstrating the feasibility of direct amplification with the QIAGEN® Investigator® 24plex GO! Kit for the processing of reference samples collected on non-treated matrices. Attendees will also be presented kit characteristic data including average peak heights and inter- and intra-color balance values.

Recent trends in sample processing for databasing and paternity purposes have moved toward direct amplification systems. DNA samples can be collected and stored on non-treated matrices, such as the Bode Buccal DNA Collector™ until sample testing is required. QIAGEN's® recently released direct amplification kit encompasses the Combined DNA Index System (CODIS) core loci, the European standard set markers, and additional loci including a Y-chromosomal Short Tandem Repeat (Y-STR) and quality sensors.

This presentation will describe the studies performed with the Investigator® 24plex GO! Kit to obtain optimal results from samples collected utilizing the Bode Buccal DNA Collector™, a non-treated matrix, at varying reaction volumes: 20µl (Full Reaction), 10µl (Half Reaction), and 5µl (Quarter Reaction). A total of 100 (*n*=100) self-collected samples, approximately 1.5 years old at the time of testing, were utilized in this experiment. These 100 samples were stored in a controlled micro environment (~20°C-25°C and <10% humidity). Slightly aged samples were chosen as they may be more representative of a routine databasing sample rather than a fresh sample collected a few days prior to testing.

This presentation will display the optimized procedures for cell lysis, reaction mix components, thermal cycling parameters, and 3500xL injection conditions. The manufacturer's recommended procedure for "other papers" or non-treated matrices did not include a cell lysis step. The use of this procedure resulted in a poor success rate with very few samples exhibiting called alleles. A modified procedure was developed in order to perform a direct amplification procedure with a 1.2mm punch from a non-treated matrix. 2µl of QIAGEN's® Investigator® STR GO! Lysis Buffer (designed for a swab protocol) was added to a 1.2mm punch contained in a 96-well reaction plate. After ensuring that the punch was submerged in the liquid, the reaction plate was placed on a heat block set at 95°C for five minutes in order to dry the punch. Failure to incubate the sample and dry the punch resulted in DNA profiles with a ski slope, and the complete dropout of the "S" internal quality sensor indicating inhibition.

The volumes of the amplification reaction mix components followed the manufacturer's recommendations for the full (20µl 27 cycles) reaction. Each component was proportionally reduced to create the half (10µl 26 cycles) and quarter (5µl 25 cycles) reaction mixes.

Capillary electrophoresis setup and run parameters were optimized to achieve consistent, reliable, and reproducible results. The manufacturer's recommendation indicated to add 12µl of formamide/Internal Lane Standard (ILS) mix containing 12µl of formamide and 0.5µl of BTO550 ILS per sample. This resulted in low overall ILS peak heights (~300-500 Relative Fluorescence Units (RFUs)). Multiple samples failed as relatively low pull-up peaks (~100-200 RFUs) caused significant sizing issues resulting in uninterpretable data. Optimization parameters, including the use of additional ILS and a standard amplification product dilution, will be discussed during the presentation.

These optimized procedures resulted in a first pass success rate of 93% for a full reaction (20µl). The highest first pass success rate (99%) was observed when utilizing the half reaction (10µl) protocol. In the full reaction, the "S" quality sensor displayed RFU values, on average, slightly higher than the "Q" quality sensor. In the half reaction, the opposite was observed.

Direct amplification of reference samples utilizing QIAGEN's® Investigator® 24plex GO! Kit can provide a time-efficient method for obtaining complete genetic profiles with a high first pass success rate. This presentation will demonstrate methods to increase direct amplification feasibility by decreasing overall costs per sample through the use of reduced volume reactions.

Direct Amplification, QIAGEN®, Buccal Sample

B116 Increasing DNA Mixture Analysis Quality and Efficiency

George R. Riley, PhD, National Center Biotechnology Information, National Institutes of Health, 45 Center Drive, Bethesda, MD 20892; Robert M. Goor, PhD, Natl Ctr Biotechnology Information, National Institutes of Health, 45 Center Drive, Bethesda, MD 20892; Douglas Hoffman, MS, Natl Ctr Biotechnology Information, National Institutes of Health, 45 Center Drive, Bethesda, MD 20892; and Stephen Sherry, PhD, 45 Center Drive, Bethesda, MD 20892-6513*

After attending this presentation, attendees will better understand the challenges of distinguishing low-level artifacts from low-level alleles and how expert software can help distinguish between them to increase analyst efficiency and mixture profile interpretation reproducibility.

This presentation will impact the forensic science community by increasing awareness of how free, open-source software can be used to improve the significant problem of reproducibility in the interpretation of mixed DNA profiles.

Forensic DNA laboratories are attempting to find analytical thresholds that utilize the greatest amount of information possible in their Short Tandem Repeat (STR) mixture profiles without mistaking small artifacts and noise for alleles. Simultaneously, backlogs create pressure to use analyst time efficiently, while increased sensitivity increases the need for more reproducible interpretation.[1] The Scientific Working Group on DNA Analysis Methods (SWGDAM) Interpretation Guidelines for autosomal STR typing indicate that laboratories should not use their analytical threshold to avoid artifacts, but rather should apply analytical thresholds that take the laboratory's empirical noise levels into account. This allows laboratories to utilize the maximum information in STR profiles, and not "leave data on the table." As laboratories begin to use analytical thresholds below 30 Relative Fluorescence Units (RFU) to analyze complex mixed profiles, it becomes critical to be able to distinguish actual alleles from small STR artifacts and excursions in the baseline noise. STR analysis noise arises from Polymerase Chain Reaction (PCR) amplification artifacts and noise in the capillary electrophoresis analyzer. With more sensitive analytical thresholds, analysts spend more time determining whether low-level peaks in mixtures are alleles or artifacts. Software can employ various mathematically determined metrics to identify and discriminate low-level artifacts from alleles.

The Open Source Independent Review and Interpretation System (OSIRIS), downloadable from the National Center for Biotechnology Information (NCBI) OSIRIS homepage, was created in response to recommendations arising from the World Trade Center victim identification effort. OSIRIS is in use as an expert system for Combined DNA Index System (CODIS) samples and in clinical and forensic caseworking laboratories to analyze complex and low-level mixtures. OSRIS implements unique STR analysis metrics and a variety of artifact signatures that give the software exceptional capabilities when analyzing low-level STR profiles.

OSIRIS computes novel metrics for peak shape, peak shifting, sample-to-ladder fit, and channel-specific baseline noise. OSIRIS also matches various mathematical artifact signatures to different peak shapes and applies these metrics and signatures to discriminate allele peaks from artifacts and noise. Low-level artifacts include pull-up, non-specific peaks and random deviations in baseline noise that can appear to be alleles. Low-level alleles include those masked as shoulders on larger alleles and can be mistaken for artifacts or noise. Low-level artifact peaks can be difficult to visually distinguish from allele peaks. OSIRIS uses its calculated metrics, peak signatures, and its expert knowledge base to automatically identify and annotate these and other artifacts, saving significant analyst time and enhancing reproducibility among analysts.

Accurately and robustly identifying these artifacts increases the assurance of quality profiles and reduces both the editing burden and the number of conflicting analyst/reviewer calls that require resolution. In this way, OSIRIS improves the efficiency of analysts that are interpreting profiles. This gives analysts more time to do the important work of case interpretation.

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DNA, Mixture, Reproducibility

B117 Using Bayesian Networks for the Interpretation of Low-Template DNA Profiles at the Activity Level

Ka-Man Pun, Polizia Cantonale - Scientifica, via Chicherio 20, Bellinzona, Ticino 6500, SWITZERLAND; and Christophe Champod, PhD, Batochime, Lausanne 1015, SWITZERLAND*

After attending this presentation, attendees will understand how to interpret low-template DNA profiles at the activity level using a graphical model and understand more about phenomena like transfer, background, persistence, and contamination.

This presentation will impact the forensic science community by changing the interpretative approach about DNA evidence generally based on source level propositions.

Today, a very limited quantity of epithelial cells deposited on an object can yield to a DNA profile. Such profiles are known under the term low-template DNA. Such sensitivity has opened new interpretative issues, principally because of the uncertainties regarding the nature of the biological material involved, the relevancy of the evidentiary material, and the representativeness of the DNA profile obtained. Interpreting DNA evidence arising from low-template DNA is a challenge yet to be solved. Significant contributions have been made when the case is assessed at the "source" level. Some researchers have started to investigate some of the factors that impact the trace level deposition and the profile quality. It is fair to say that very little is known about combining these variables into a coherent interpretative model and moving the interpretation of these cases from the "source" level to the "activity" level; however, "activity" level propositions should be presented in order to help the court understand the significance of the forensic findings within the context of a criminal case.

When considered in this context, the likelihood ratio reflects more than the mere discriminatory power of the DNA. The likelihood ratio also depends on different phenomena: transfer, persistence, contamination, and background. Each of these is influenced by the others. For example, the transfer phenomenon can be affected by the shedder's quality, the substrate's quality, and the nature of the contact; on the other hand, the persistence is dictated by the substrate quality, exogenous factors (i.e., temperature, humidity rate, light exposure, etc.), and the time between the evidence deposition and collection.

The purpose of this doctoral research is to design a graphical model (in the form of Bayesian networks) for solving the probabilistic relationships between parameters that are of relevance to the interpretation of a low-template DNA recovered during a police investigation. The model allows identifying the datasets that are the most relevant to the task at hand. By doing this, it is possible to focus the experimental data-gathering process only on the factors that play a major role. Then the model (appropriately informed with acquired data) allows an assignment of likelihood ratios at an activity level in a range of scenarios. In the end, a sensitivity analysis shows the robustness of the proposed model even in the presence of uncertainty in this forensic field.

Vehicle thefts have been selected from among the various forensic scenarios. This scenario presents several advantages compared with the others. First, different types of substrate are present in a car; second, the presence of the victim (the car owner) can be assumed/integrated into the graphical model.

According to the results, the factors having the strongest impact on the likelihood ratio are transfer and the background. These phenomena can also significantly affect the evaluation according to case-specific propositions.

Interpretation, DNA Evidence, Bayesian Networks

B118 2016 Update From the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG)

Sandra E. Rodriguez-Cruz, PhD, Drug Enforcement Admin, Southwest Laboratory, 2815 Scott Street, Vista, CA 92081*

After attending this presentation, attendees will better understand the latest documents and resources developed by SWGDRUG.

This presentation will impact the forensic science community by informing attendees of current activities, including revisions to the SWGDRUG recommendations, development of new Supplemental Documents, and other resources available to the seized-drug community.

SWGDRUG was formed in 1997 in a joint effort between the United States Drug Enforcement Administration (DEA) Office of Forensic Sciences and the Office of National Drug Control Policy (ONDCP). SWGDRUG works to improve the quality of the forensic examination of seized drugs and to respond to the needs of the forensic community by supporting the development of internationally accepted minimum standards, identifying best practices within the international community, and providing resources to help laboratories meet these standards.

During the summer of 2015, the SWGDRUG core committee started revising Part IIIB and Part IVB of the SWGDRUG Recommendations. These sections pertain to the categorization of analytical techniques and the validation of analytical methods, respectively. The development of new instrumental technologies and their introduction to seized-drug laboratories have brought up the need to clarify the categorization of techniques into A, B, or C groups. Revisions to SWGDRUG Recommendations Part IIIB will not involve changes in these categories, but will focus on providing guidance regarding the different modes of operation and variations within a technique and how their categorization could be affected.

Revisions to Part IVB incorporate clarifications on the performance characteristics that should be evaluated during the validation of qualitative and quantitative methods. Also, examples of method validation schemes for routinely used techniques such as color test, Gas Chromatography/Mass Spectrometry (GC/MS), and Infrared (IR) spectroscopy will also be included and made available to users in the form of a supplemental document.

SWGDRUG committee members are also in the process of developing Supplemental Document 6 (SD-6) titled "Examples of Measurement Uncertainty for Net Weight and Count Extrapolations." This document will assist seized-drug analysts by providing step-by-step examples for estimating uncertainty for scenarios where the net weight of an exhibit is obtained via extrapolation or when the total count of a dosage unit exhibit needs to be extrapolated.

This presentation will also summarize recent updates on some of the resources provided by SWGDRUG such as the MS library, the IR library, and the Drug Monographs. Laboratory analysts throughout the world can download the libraries from the SWGDRUG website and onto their laboratory instruments. Currently, the MS library contains more than 2,200 spectra, including many of the recently encountered synthetic cannabinoids, bath salt compounds, and their isomers. The IR library contains more than 150 reference materials which have been structurally characterized by the DEA Special Testing and Research Laboratory. More than 260 Drug Monographs are also available for use during reference material verifications.

The SWGDRUG core committee is comprised of representatives from federal, state, and local law enforcement agencies in the United States, Canada, Brazil, Great Britain, Germany, Austria, Switzerland, Australia, and Singapore. The following forensic organizations are represented: the European Network of Forensic Science Institutes (ENFSI), the Academia Iberoamericana de Criminalistica y Estudios Forenses (AICEF), the Asian Forensic Science Network (AFSN), and the United Nations Office on Drugs and Crime (UNODC). Core committee members also include forensic science educators and representatives from forensic science organizations across the United States, the American Society of Crime Laboratory Directors (ASCLD), the American Society for Testing and Materials (ASTM), and the National Institute of Standards and Technology (NIST).

Criminalistics, SWGDRUG, Seized Drugs

B119 Analysis and Extraction of Fentanyl in Seized Heroin Samples

Charles A. Richardson-Gongora, 19360 NW 10th Street, Pembroke Pines, FL 33029; Michael M. Healy, MBA, Manatee County Sheriff's Office, 600 301 Boulevard, W, Ste 202, Bradenton, FL 34205-7953; and Gerald Mattson, PhD, University of Central Florida Chemistry Department, 4000 Central Florida Boulevard, Orlando, FL 32816*

The goal of this presentation is to create an extraction procedure which can isolate fentanyl and identify compounds from heroin samples.

This presentation will impact the forensic science community by presenting a proposed extraction procedure that will assist in the determination of various fentanyl compounds present in heroin samples; this will prove helpful as fentanyl-laced heroin samples are being encountered with higher frequency.

The availability and demand of heroin has increased in the United States due to abusers switching from controlled prescription drugs to heroin.[1] Many factors may have contributed to this such as heroin's affordability, higher potency, and availability. These circumstances can lead to greater predicaments when individuals desire a stronger euphoria, thereby creating a demand for adding designer drugs to heroin. Though fentanyl is a Schedule II controlled substance, its readily synthesizable analogues are either Schedule I or not scheduled at all. As a result, issues in laboratory analyses can arise from similarities found in mass spectra and the lack and/or differing of scheduling pertaining to some analogues. Some consider fentanyl a "designer drug," regardless of its prevalence in medical practice. According to the Drug Enforcement Agency, some analogues may be considered as "controlled substance analogs"; however, "any substance…not intended for human consumption…" is considered an "exemption," allowing them to circumvent the law.[2]

This study is a response to an increase of fentanyl analogues (fentanyl-types) in seized heroin samples appearing in Manatee County, FL. Within the past two years, Manatee County has experienced an increase in heroin usage.[3] The two most encountered fentanyl-types were discovered to be acetylfentanyl and β-hydroxythiofentanyl. Acetylfentanyl, a known impurity of fentanyl synthesis, was normally found accompanied by fentanyl.[4] Currently, it is being considered for temporary scheduling by the Department of Justice as a Schedule II designer drug. The other analogue, β-hydroxythiofentanyl, was seen often accompanied by dimenhydrinate (Dramamine®) or cetirizine, possibly to counteract undesired effects and to lower fentanyl potency. The presence of β-hydroxythiofentanyl is of concern because of its lack of scheduling in the state of Florida and similar mass spectrum to fentanyl.

β-hydroxythiofentanyl produces a similar mass spectrum to fentanyl and therefore needs additional confirmation such as retention-time comparison. The identification of acetylfentanyl can be difficult owing to similarities to acetylfentanyl-4-methylphenethy; however, a standard was not procured and no retention time comparisons could be made. Instead, Attenuated Total Reflectance/Infrared (ATR/IR) spectrophotometry was conducted on extracts of confirmed heroin samples containing fentanyl-types. Extractions were conducted using a liquid extraction method based upon fentanyl and heroin solubility and an online purification method.

The extracts were analyzed first using Gas Chromatography coupled with Mass Spectrometry (GC/MS) to confirm the success of an extraction and then using ATR/IR spectroscopy to determine final composition of the compound. The method was successful in isolating the fentanyls; however, extractions were unable to separate fentanyl-types from each other or other cutting agents. The concomitants did not pose any issues due to lower concentrations than the target analogue. The analysis of five extracts and 406 chromatographs of seized heroin samples qualitatively confirmed that the main analogues present in Manatee County were acetylfentanyl and β-hydroxythiofentanyl in lieu of other analogues

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Fentanyl, Fentyl Analogues, Heroin

B120 Analysis of Prescription Drugs With Abuse-Deterrent Properties

Robert P. Bianchi, BS, 12728 Penguin Drive, Bradenton, FL 34212*

After attending this presentation, attendees will better understand the analysis of drugs with abuse-deterrent properties.

This presentation will impact the forensic science community by improving the efficiency of controlled drug analysis.

Prescription drug abuse is sweeping the nation faster than ever before. In 2012, an estimated 2.4 million Americans used prescription drugs non-medically for the first time within the past year, which averages approximately 6,700 new users per day, according to the 2012 National Survey on Drug Use and Health.[1] Prescription drug abuse is the non-medical use of a medication without a prescription, in a way other than as prescribed, or for the experience or feelings elicited.

In 2013, an estimated 24.6 million or 9.4% of the American population aged 12 or older were currently illicit drug users. Marijuana remains the most commonly used illicit drug with 19.8 million users (more than heroin and cocaine combined). Non-medical use of prescription drugs is the second-largest category of abused drugs at 6.5 million users or 2.5% of the population. Centers for Disease Control and Prevention classified this phenomenon as an epidemic.

The classes of prescription drugs most commonly abused are: opioid pain relievers, such as Vicodin® or OxyContin®; stimulants for treating Attention Deficit Hyperactivity Disorder (ADHD), such as Adderall®, Concerta®, or Ritalin®; and Central Nervous System (CNS) depressants for relieving anxiety, such as Valium® or Xanax®.

The pharmaceutical industry and government agencies have embarked on an initiative to reduce prescription drug abuse by making the formulations more difficult to abuse. The advent of abuse-deterrent formulations is creating new challenges for the forensic community. Abuse-deterrent formulations of interest have in part been categorized by the Food and Drug Administration (FDA) as follows: (1) Physical/chemical barriers — Physical barriers can prevent chewing, crushing, cutting, grating, or grinding of the dosage form. Chemical barriers, such as gelling agents, can resist extraction of the opioid using common solvents like water, simulated biological media, alcohol, or other organic solvents. Physical and chemical barriers can limit drug release following mechanical manipulation or change the physical form of a drug, rendering it less amenable to abuse. (e.g., reformulated OxyContin®); (2) Agonist/antagonist combinations — An opioid antagonist can be added to interfere with, reduce, or defeat the euphoria associated with abuse. The antagonist can be sequestered and released only upon manipulation of the product; (3) Aversion — Substances can be added to the product to produce an unpleasant effect if the dosage form is manipulated or is used at a higher dosage than directed; and, (4) New molecular entities and prodrugs — The properties of a new molecular entity or prodrug could include the need for enzymatic activation, different receptor binding profiles, slower penetration into the central nervous system, or other novel effects.

These new formulations are designed to make extraction by abusers more difficult; however, they also make it more complicated for analysts in a laboratory. Forensic chemists have always been challenged by prescription drug exhibits and the advent of abuse-deterrent formulations makes analysis more difficult. Heroin, cocaine, and methamphetamine exhibits are routine and easier to characterize, while abuse-deterrent prescription drugs present unique challenges. The presence of certain ingredients will dictate how the analysis should be approached. For example, the presence of Polyethylene Oxide (POLYOX), which is used as a binding, thickening, or water-retention agent, will inhibit solvent extraction. Analysts should determine the ingredients contained in a prescription drug by visiting internet sites or published dosage form descriptions such as http://www.drugs.com/pill-logo-identification.html, http://www.rxlist.com/pill-identification-tool/article.htm, or the *Physician's Desk Reference*. Once the formulation is known, then the analytical approach can be developed. This is especially important if there is a legal requirement to report the amount of controlled substance present.

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Abuse Deterrent, Prescription Drugs, Forensic Analysis

B121 The Prevalence of Promethazine Dimerization in Forensic Samples of "Purple Drank"

Tyler Williams, 98 Hearthstone Way, Hanover, MA 02339; James T. Miller, BS, Houston Forensic Science Center, 1200 Traves, 20th Fl, Houston, TX 77002; and Glen P. Jackson, PhD, West Virginia University, Dept of Forensic and Investigative Science, 208 Oglebay Hall, Morgantown, WV 26506-6121*

After attending this presentation, attendees will understand how exposure to Ultraviolet (UV) light can cause the formation of a promethazine dimer.

This presentation will impact the forensic science community by providing an explanation for the presence of additional chromatographic peaks with promethazine-like mass spectra in seized samples containing promethazine.

In certain parts of the country, codeine cough syrups are consumed as a recreational drug known as "purple drank." One of the other main components in this cough syrup is an antihistamine and antiemetic drug called promethazine. When "purple drank" beverages are collected and analyzed by forensic laboratories, they often find two or more chromatographic peaks with indistinguishable promethazine-like Electron Ionization (EI) mass spectra. One of these peaks has a Gas Chromatography (GC) retention time equal to standard promethazine, whereas a second GC peak can elute more than a minute later, with an indistinguishable mass spectrum. This study was conducted to help identify the cause and identity of the second mystery peak.

Samples of promethazine were dissolved in chloroform at 50ppm and analyzed via Gas Chromatography/Mass Spectrometry (GC/MS) to determine the origin of the second peak. Initial experiments replicated the Standard Operating Procedure (SOP) of an American National Standards Institute-American Society of Quality (ANSI-ASQ) National Accreditation Board/ Forensic Quality Services (ANAB/FQS) -accredited crime laboratory for the extraction and GC/MS analysis of aqueous samples suspected to contain promethazine and codeine. Standard promethazine was dissolved in water to simulate the cough syrup, and aliquots were made basic using sodium hydroxide or a saturated sodium carbonate solution and extracted into chloroform. Neither of these caused the appearance of the second peak. The samples were also made acidic using hydrochloric acid and extracted into chloroform, but these results did not show any additional peaks besides the normal promethazine peak.

The core/base structure of promethazine is phenothiazine, and it has been found to form radicals when exposed to UV light. For this reason, the possibility of UV-induced chemistry of this phenothiazine derivative was explored. Samples of promethazine were dissolved in chloroform and were left under long-wave or short-wave UV light for different times. Upon examination using the GC/MS, the long-wave UV exposure for two or four hours showed no additional peaks, but the sample exposed to short-wave UV for four hours showed a new small GC peak more than a minute later than the promethazine peak, which was also readily abundant. Additional studies were then conducted to determine the cause of the "new" peak. One aliquot of dissolved promethazine was placed on a windowsill for two days, one was placed under short-wave UV light for seven hours, one was placed on a desktop (away from an outside window) for two days, and one was placed in the dark for two days. The samples in the dark and on the desk showed no sign of the second peak. The sample left on the windowsill and the sample left under short-wave UV light for seven hours showed that more than 50% of the promethazine had either oxidized or converted to the dimer form. Additional Nuclear Magnetic Resonance (NMR) studies were also conducted to confirm the dimerization product.

In conclusion, this study provides evidence that upon exposure to UV light, promethazine will form degradation products, which include a dimerized form of the drug. This additional peak could presumably be avoided by storing casework in amber vials or otherwise limiting the exposure to UV radiation.

Drug Chemistry, Gas Chromatography, Promethazine

B122 Adulteration of Psychoactive Herbal Supplements Revealed by Direct Analysis in Real-Time Mass Spectrometry (DART®-MS)

Ashton D. Lesiak, University of New York at Albany, Dept of Chemistry, 1400 Washington Avenue, Albany, NY 12222; Robert B. Cody, PhD, JEOL USA, Inc, 11 Dearborn Road, Peabody, MA 01960; Masaaki Ubukata, PhD, JEOL USA, Inc, 11 Dearborn Road, Peabody, MA 01960; and Rabi A. Musah, PhD, State University of New York at Albany, Dept of Chemistry, 1400 Washington Avenue, Albany, NY 12222*

After attending this presentation, attendees will understand the value of Direct Analysis in Real-Time Mass Spectrometry High Resolution Mass Spectrometry (DART®-HRMS) for rapid confirmation of the identity of psychotropic plant material and for screening psychoactive herbal supplements for banned substances.

This presentation will impact the forensic science community by demonstrating the benefit of DART®-HRMS in drug chemical analysis, especially in terms of the speed and simplicity of analysis of samples in complex matrices. Definitive identification of psychoactive compounds can be accomplished using in-source Collision-Induced Dissociation (CID) and screening for adulterants can be rapidly performed. The ability to characterize and identify not only unscheduled plant-based psychotropics but also controlled substances could assist in reducing casework backlogs in forensic drug laboratories.

Sceletium tortuosum, commonly known as Kanna, is a psychotropic plant that has been identified by the United Nations Office on Drugs and Crime as a drug of concern.[1,2] Historically used for medicinal purposes, Kanna is marketed as an antidepressant, stimulant, and a natural alternative to traditional drugs of abuse.[2,3] Kanna is often sold as an herbal supplement and is found in multiple incarnations (seeds, powders, resin, etc.), which makes it challenging to identify. Because of its classification as a dietary or herbal supplement, Kanna is exempted from mandatory regulation and testing by the United States Food and Drug Administration (FDA). Consequently there is little oversight regarding the ingredient profile of Kanna products.

There have been many reported cases of herbal supplements being laced with toxic or banned substances.[4-6] The sample matrices are often complex, which makes analysis and definitive identification of these substances using conventional methods quite challenging. Hyphenated methods such as Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Mass Spectrometry (LC/MS) can be used to determine supplement identity and reveal the presence of adulterants; however, these approaches often require tailored method development that targets specific analytes of interest. With the increasing numbers of psychoactive herbal supplements on the market, it is impractical to develop standard operating protocols for each of these unscheduled substances.

DART®-HRMS is an ambient ionization method that can circumvent some of the aforementioned challenges in plant-based sample analysis. DART®-HRMS screening of Kanna samples revealed that these herbal products exhibit characteristic chemical fingerprints that are consistent with the presence of psychoactive and chemotaxonomic markers previously detected in the *S. tortuosum* species. These include mesembrine, mesembrenol, mesembrenone, and hordenine. Comparison of the in-source CID spectra of available authentic standards to in-source CID spectra of the Kanna products confirmed the presence and identity of the biomarker hordenine. Furthermore, the results of the in-source CID analysis revealed the presence of ephedrine in one of the herbal supplements. This finding was independently confirmed by GC/HRMS analysis.

The identification of ephedrine as an adulterant in Kanna products demonstrates the benefit of using DART®-HRMS for screening of psychoactive plant material and herbal supplements, as many traditional analysis methods would have been unlikely to have revealed its presence. DART®-HRMS was shown to be a powerful tool to not only screen plant-based drugs of abuse for psychotropic alkaloids, but also to reveal the presence of banned substances and adulterants.

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Legal Psychoactives, Ephedrine, Adulterated Supplements

B123 Toward On-Site, Real-Time, Confirmatory Analysis of Drugs and Their Optical Isomers Using a Battery-Operated, Portable, Ultra-Fast Capillary Electrophoresis/Mass Spectrometry (UFCE/MS)

Mehdi Moini, PhD, George Washington University, Dept of Forensic Sciences, 2100 Foxhall Road, NW, Washington, DC 20007; and Christopher M. Rollman, BS, George Washington University, 2100 Foxhall Road, NW, Somers Hall, L14, Washington, DC 20007*

After attending this presentation, attendees will better understand a novel portable device for on-site, real-time confirmatory analysis of drugs and their optical isomers.

This presentation will impact the forensic science community by discussing the role of a portable confirmatory technique for the detection of explosives, illicit drugs, and their optical isomer that takes approximately one minute.

In the past several years, there has been considerable interest in developing hand-held devices for detection of chemicals and biological samples in the forensics area. Most of the devices are based on spectroscopic techniques and are therefore only sufficient for screening purposes. To address the need for portable confirmatory methods, a variety of portable mass spectrometers have been developed and used with real-time ionization techniques such as Desorption Electrospray Ionization (DESI), Direct Analysis In Real Time (DART®), and paperspray ionization. The limited or lack of separation with these ionization techniques makes the analysis of complex mixtures difficult; therefore, a portable and fast separation technique is desired. A variety of fast separation techniques based on Gas Chromatography (GC), Liquid Chromatography (LC), supercritical fluid chromatography, and Capillary Electrophoresis (CE) have been developed, but CE is the most amenable to miniaturization. Presented here is a portable (battery-operated), UFCE/MS for fast separation of drugs and their optical isomers.

Analyses were performed using fused silica capillaries utilizing a porous tip to interface between the CE and MS. Short (≤25cm long) capillaries with inner diameters between 5µm and 15µm were used for separation. The capillary wall was coated using polybrene, which under reverse polarity mode provided electro-osmotic flow toward the outlet. A separation voltage of -25kV was used to achieve ≥1,000V/cm across the capillary. A background electrolyte analyses of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (18-C-6-TCA) was used.

The portable UFCE electrospray ionization source fits in front of a low- or high-resolution MS similar to a conventional electrospray/nanospray ionization source and has about the same size and weight. The UFCE ionization source is equipped with two high-voltage power supplies (±25kV HVPS) capable of operating in forward or reverse polarity modes and powered by a 12V rechargeable Lithium ion battery, making it compatible with a portable MS. The device was able to separate illicit drugs (including amphetamines, cathinones, and cannabinoids) and their optical isomers in approximately one to two minutes. The portable ultrafast CE ionization source for MS allows for separation of compounds and their isomers in about one minute.

Portable CE, CE/MS, Explosive Drugs Optical Isomer

B124 Single Crystal X-Ray Diffraction in Forensic Drug Analysis

Matthew R. Wood, MS, Ocean County Sheriff's Dept, Forensic Science Laboratory, Toms River, NJ 08753; Thomas A. Brettell, PhD, Cedar Crest College, 100 College Drive, Allentown, PA 18104; Ivan Bernal, PhD, Rutgers University - Newark, 73 Warren Street, Newark, NJ 07102; Hugh W. Thompson, PhD, Rutgers University - Newark, 73 Warren Street, Newark, NJ 07102; and Roger A. Lalancette, PhD, Rutgers University - Newark, 73 Warren Street, Newark, NJ 07102*

After attending this presentation, attendees will better understand the crystal structure of the precipitate resulting from the gold chloride microcrystal test with ecgonine, several phenethylamines, and 3,4-methylenedioxypyrovalerone (MDPV). Additionally, attendees will understand the crystal structure of several cathinones ("bath salts") and the additional information that X-ray diffraction can provide to the analyst. This presentation will describe how these structures were determined using single crystal X-ray diffraction techniques and the potential implications for the microcrystal testing of other drugs.

This presentation will impact the forensic science community by providing essential information regarding the molecular structure and interactions within the crystal lattice of the compounds investigated as well as the benefits of single crystal X-ray structure determination.

The analysis of controlled dangerous substances by microcrystal tests has been largely empirical, relying on the experience and training of the analyst to make conclusions regarding the identity of microcrystalline precipitates viewed microscopically. The microscopist had little support beyond the optical characteristics provided by the polarized light microscope to base his/her conclusions. While the morphology and optical properties of the resulting crystals have been studied and documented, very little structural information has been available on the molecular level. Single crystal X-ray diffraction is an ideal technique for determining the chemical composition of crystals containing highly diffractive heavy metals, such as gold chloride complexes.[1]

Ecgonine precipitates as crystals with the gold chloride reagent as both the anhydrous salt and the hydrate. Single crystals of ecgonine-gold chloride was grown for comparison to previous work with cocaine-gold chloride.[2] For the hydrate, the asymmetric unit consists of four protonated cations of ecgonine, surrounded by five gold chloride anions and seven waters, one of which is a hydronium ion for charge balance. The crystal lattice contains a network of water molecules woven between the cations and anions making hydrogen bonds with the hydroxyl and carboxyl functions of the ecgonine molecules. The anhydrous crystal of the ecgonine salt demonstrates close contact bonding between the gold chloride anions and the ecgonine cations and an intramolecular hydrogen bond between the protonated nitrogen and the carboxyl oxygen.

A series of related phenethylamines (ephedrine, amphetamine, and methamphetamine) were grown with gold chloride as single crystals for X-ray diffraction. Both ephedrine and methamphetamine precipitated as salts with the 1:1 ratio of cation-to-anion as seen with other microcrystal tests. The ephedrine-gold chloride complex shows a stratified internal arrangement in which the phenyl rings of the ephedrine cations are in bands every one-third of the unit cell and the gold chloride anions reside in clusters near the protonated N atoms of the cations.

However, amphetamine produced a unique cycloaurate complex with a centrally located Au atom bound bidentate to the amphetamine ligand and to two Cl atoms. In the methamphetamine-gold chloride crystal structure, the gold chloride anion is aligned between the phenyl rings of the cations, deviating from a line drawn between the centroids of the phenyl rings by less than 0.6Å.

The "bath salt" MDPV was studied as the gold chloride precipitate. A single hydrogen bond exists from the protonated N atom of the cation to the proximal anion. Additionally, several law enforcement seizures of crystal MDPV were examined directly by X-ray crystallography as submitted to the laboratory. The analysis of these samples has provided some insight into the nature of these dangerous compounds.

The purpose of this research is to gain an understanding of how the structure of the selected compounds and their gold (III) chloride complexes may play a role in the formation of crystals visualized by the traditional microcrystal test. This is an ongoing research project at Rutgers University. Further crystallographic studies will focus on other related drug compounds and additional microcrystal tests.

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Drug Analysis, Crystallography, Microcrystal Tests

B125 Microcrystalline Tests in Conjunction With Vibrational Spectroscopy for the Analysis of Illicit Drugs and Their Metabolites

Brooke W. Kammrath, PhD, University of New Haven, Forensic Science Dept, 300 Boston Post Road, West Haven, CT 06516; Shannon Tilly, 41 William Street, Fair Haven, NJ 07704; Kara Kovacev, 53 Riverbank Road, Saugus, MA 01906; Natasha L. Kuegler, BS, University of New Haven, Forensic Science Dept, 300 Boston Post Road, West Haven, CT 06516; Pauline E. Leary, PhD, Smiths Detection, 1934 Bulls Head Road, Stanfordville, NY 12581; and John A. Reffner, PhD, John Jay College of Criminal Justice, 524 W 59th Street, New York, NY 10019*

After attending this presentation, attendees will understand that the coupling of microcrystalline tests with vibrational spectroscopy (either infrared or Raman) is an excellent analytical scheme for drug identification because it is rapid, reliable, and creates a reviewable record of the analysis.

This presentation will impact the forensic science community by presenting a method of drug analysis that pairs microcrystalline tests with vibrational spectroscopy, thus creating an analytical scheme that satisfies the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) standards and can be easily incorporated into a criminalist's drug-testing protocol.

The analysis and identification of illicit drugs is a critical component of the field of forensic science as it constitutes a majority of casework performed in most forensic laboratories. Any analytical method for the identification of drugs must be rapid, reliable, and create a reviewable record in order to satisfy legal standards as well as manage the large number of submissions to a forensic laboratory. SWGDRUG has established minimum standards for the forensic identification of illicit drugs.[1] The SWGDRUG guidelines classify techniques into three categories which are based on discriminating power, with A being the most and C the least. One proposed analytical scheme is using two techniques, one of which must be from category A (infrared spectroscopy, Raman spectroscopy, mass spectrometry, nuclear magnetic resonance spectrometry, or X-ray diffractometry). This research proposed the coupling of microcrystalline tests (a Category B technique) with vibrational spectroscopy (either infrared or Raman) as a fast, efficient, inexpensive, and non-destructive drug analysis and identification analytical scheme that satisfies SWGDRUG standards and can be easily executed by criminalists.

Microcrystalline tests are highly developed precipitation tests that use specific reagents to create crystals with particular morphologies that can be used to determine the presence of many chemicals, including both controlled substances and other related compounds. An advantage of microcrystalline testing is that there are a plethora of crystal habits that show morphological differences, and this enables closely related analogs to be readily differentiated. In addition, these tests work with sample mixtures and, in effect act, as a method of separation because the reagent will form characteristic microcrystals only with specific drugs. Although in the past microcrystalline tests were considered to be confirmatory techniques, today they are used as preliminary or presumptive testing methods to indicate what the drug might be, which is why instrumentation is needed to confirm the results. Infrared and Raman spectroscopy are common forms of instrumentation that are used in forensic laboratories to identify illicit drugs. Although both types of vibrational spectroscopy are considered to generate the highest discriminating capabilities, the discrimination power is considered to be diminished in mixtures because what is produced is a combined spectrum that is more difficult to interpret. Thus, a method of separation is often employed to make a pure sample capable of spectroscopic analysis.

The proposed method uses microcrystalline tests both to identify the drug by its morphology and also as a means for isolating the drug for vibrational microspectral analysis. Prior research published by Wielbo and Tebbett coupled infrared microspectroscopy with microcrystalline tests of seven drugs; however, the microcrystalline samples were allowed to dry at room temperature prior to being analyzed by infrared spectroscopy.[2] The current research improves on this method by using a diamond-Attenuated Total Reflection (d-ATR) infrared microprobe, thus enabling analysis of the microcrystals in solution.[3] Research presented at the 2007 American Academy of Forensic Sciences (AAFS) Annual Scientific Meeting by Cullinan and Bell used both Raman and infrared microspectroscopy to analyze drug microcrystals of phenylethylamines (amphetamine, methamphetamine, phentermine, and ephedrine).[4] This research has extended the application of this technique to the identification and confirmation of cocaine and its metabolite ecognine, morphine, codeine, as well as additional phenylethylamines, such as common bath salts (methylenedioxypyrovalerone or MDPV and ethylone) and 3-4-methylenedioxymethamphetamine (MDMA).

The coupling of microcrystalline tests with vibrational spectroscopy is a rapid testing method which requires minimal reagents, combines two reliable techniques, produces reviewable records of the results (in the form of photomicrographs and spectra), is non-destructive (test compounds are recoverable from the test slide), uses instrumentation that is currently available in most forensic laboratories, and satisfies SWGDRUG guidelines for the identification and confirmation of illicit drugs. This research demonstrated that pairing microcrystalline tests with either infrared or Raman microspectroscopy is a valuable technique for drug identification within the ever-growing field of forensic drug analysis.

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Drugs, Microcrystalline Tests, Spectroscopy

B126 Using Climate Modeling to Predict the Origin of Seized Cannabis

Jurian A. Hoogewerff, PhD, National Centre for Forensic Studies, Faculty ESTeM, University of Canberra, Bruce - Canberra, ACT ACT 2601, AUSTRALIA; Shaerii Sarker, MSc, Department of Chemistry, University of Otago, Dunedin 9016, NEW ZEALAND; Alan Hayman, PhD, Department Chemistry, University of Otago, Dunedin 9016, NEW ZEALAND; and Russell Frew, PhD, Department Chemistry, University of Otago, Dunedin 9016, NEW ZEALAND*

After attending this presentation, attendees will appreciate the potential of using climate data to predict the isotopic composition of natural drugs and other natural materials and the use of their spatial distribution for geographical provenancing intelligence.

This presentation will impact the forensic science community by creating awareness that it is possible to use geospatial climatic data to obtain natural drugs' provenance intelligence.

The hydrogen and/or oxygen isotopic composition of many natural biological materials is strongly dependent on the local isotopic composition of groundwater, which in is turn directly related to that of local precipitation. The Hydrogen (H) and Oxygen (O) isotopic composition of precipitation is a function of a number of factors. The cause of different relative abundances of the H and O isotopes in water molecules is the isotopic fractionation during evaporation and condensation. As the contribution of the different masses of the isotopes on the total energy of the water molecule is temperature dependent and more pronounced at lower temperatures, also more fractionation is observed at lower temperatures.

The two main factors that determine the H and O isotopic composition of precipitation at a specific location are the isotopic composition of the surface water in the source region where evaporation took place (which for major air masses is often one of the oceans) and the temperature during precipitation. Both the temperature of the source surface water and that of the air mass during precipitation will correlate with the general geographical latitude where a specific air mass is travelling. As an air mass travels inland and loses moisture through precipitation, the isotopic composition of the residual air mass will be isotopically lighter as it loses the heavier fraction in the precipitation. Often as an air mass travels inland, its altitude will increase, especially over mountainous areas, and as temperature generally drops with altitude, the isotopic composition of the precipitate also changes.

As the mentioned processes are well understood and high-resolution climatic and topographic data are freely available, it is thus possible to create spatial models that predict the isotopic composition of groundwater that is taken up by plants such as cannabis. In order to develop the critical modeling step to link the isotopic composition of groundwater with that of cannabis buds and/or leaves, many samples of cannabis of different climate zones would be required for analysis. As the seizing of cannabis by the police in New Zealand is opportunistic and not in a systematic spatial or temporal manner, it was decided to test the use of blackberry as a proxy for cannabis. Blackberry belongs to the same botanical order of Rosales and is ubiquitous in New Zealand. Blackberry was collected at 130 locations on the North and South Islands. In addition, cannabis samples from four locations at different latitudes were collected by the Institute of Environmental Science and Research (ESR) and New Zealand police for comparison and validation with the blackberry results.

Monthly water isotope data collected from 50+ locations by this group from 2007 to 2010 was combined with climate data from the New Zealand climate research organization (Virtual Climate Station Network-NIWA) to produce a model that predicts the isotopic composition of precipitation on a 5km x 5km scale for the whole of New Zealand. Subsequently, the precipitation isotope data were combined with hydrogen isotope measurements of the collected blackberry leaves and a linear relation was observed. This linear relation in turn allowed for the creation of a prediction model of the H isotope composition in blackberry leaves anywhere in New Zealand.

To validate the use of the blackberry data as a proxy for cannabis, both bulk leaf and compound-specific measurements of the hydrogen isotopic composition of both plants were compared. The initial data shows linear correlations that provide proof of principle but a larger and controlled set of cannabis samples needs to be collected to validate the use of the prediction model throughout the whole of New Zealand. In this presentation, a source apportionment tool will be presented that depicts the statistically most likely source regions for future seized cannabis samples which will provide New Zealand with strategic intelligence as to where to direct resources to detect and destroy illegally grown cannabis.

Cannabis, Climate Modeling, Isotopes

B127 Applicability of Ultra High-Performance Supercritical Fluid Chromatography (UHPSFC) as a Separation Technique for Synthetic Cannabinoids and Synthetic Cathinones

Ira S. Lurie, PhD, George Washington University, Dept of Forensic Science, 2100 Foxhall Road, NW, Somers Hall, Lower Level, Washington, DC 20007; Stephanie R. Breitenbach, BS, 165 Cross Point Drive, Owings, MD 20736; Walter F. Rowe, PhD, George Washington University, Department of Forensic Science, 2100 Foxhall Road, NW, Washington, DC 20007; Mike Hitchcock, MS, U.S. Postal Inspection Service, Forensic Laboratory Services, 22433 Randolph Drive, Dulles, VA 20104-1000; Ioan Marginean, PhD, George Washington University, 2100 Foxhall Road, NW, Somers Hall L14C, Washington, DC 20007; Stacey L. Obrien, BS, QualX Corporation, 8300 Boone Boulevard, #500, Vienna, VA 22182; and Bruce R. McCord, PhD, Florida International University, Dept of Chemistry, University Park, Miami, FL 33199*

After attending this presentation, attendees will understand some separation principles of UHPSFC, in particular the applicability to the separation of synthetic cannabinoids and synthetic cathinones. Attendees will gain insight into whether UHPSFC should be used by forensic chemists in their general analysis scheme for emerging drugs.

This presentation will impact the forensic science community by presenting a technique that has the potential to be the best chromatographic method for distinguishing between very similar solutes, such as positional isomers and diastereomers. This information could be particularly valuable in aiding the analyst in determining which controlled substance is present and to distinguish between a controlled and non-controlled emerging drug.

The recently introduced separation technique UHPSFC produces highly efficient and rapid separations performed on a new generation of analytical SFC instruments with an environmentally friendly mobile phase, containing carbon dioxide as the major component. Supercritical and subcritical carbon dioxide has properties that are intermediate between a liquid and a gas, giving it excellent diffusivity while maintaining liquid-like properties. UHPSFC, similar to high-performance liquid chromatography and ultra high-performance liquid chromatography, is advantageous for drugs that are thermally labile, polar and non-volatile solutes that are problematic for Gas Chromatography (GC) analysis. UHPSFC offers increased selectivity for very similar compounds due to interactions with the stationary phase such as hydrogen bonding, dipole and pi-pi interactions, and is particularly useful for the separation of designer drugs including synthetic cannabinoids and synthetic cathinones ("bath salts").

The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG), which is responsible for setting standards for drug analysis, does not list SFC as an approved separation technique. In order to be listed for SWGDRUG under Category B, a separation technique should not only offer reasonable separation ability but be orthogonal to accepted techniques such as GC and Liquid Chromatography (LC).

In these studies, four achiral columns, including 2-PIC, Diol, DEA, and 1-AA (1.7µm 3.0 x 100m), and three chiral columns, including AM1, CEL1, and CEL2 (2.5µm 3.0 x 150mm), were investigated for the separation of synthetic cannabinoids and bath salts using carbon dioxide with various modifiers and additives. The modifiers included methanol, acetonitrile, ethanol, and isopropanol, while the additives included ammonium formate and ammonia. Detection was carried out by photo diode array-Ultraviolet (UV) in series with single quad Mass Spectrometry (MS). The synthetic cannabinoids studied consisted of 24 controlled drugs, including a pair of positional isomers and two pairs of diastereomers, nine non-controlled positional isomers of controlled JWH-018, a non-controlled positional isomer of controlled JWH-073, and a non-controlled diastereisomer of HU-210. The "bath salts" investigated included 15 controlled drugs, with three pairs of positional isomers, seven non-controlled positional isomers of controlled pentedrone and 4-methylcathinone, four non-controlled positional isomers of controlled mephedrone and buphedrone, three non-controlled positional isomers of controlled α-PVP, two non-controlled positional isomers of controlled 4-MEPP and α-PBP, and one non-controlled positional isomer of controlled methcathinone, butylone, methylone, pentylone, and MDPV.

Although significant co-elution was observed for the controlled synthetic cannabinoids using UHPSFC, the co-eluting compounds could easily be distinguished by MS detection. Furthermore, the work demonstrates that UHPSFC is particularly valuable for the separation of isomers (both controlled and non-controlled) and all the positional and most diastereomers of the synthetic cannabinoids were resolved using this group's optimized parameters in less than ten minutes. For the "bath salts," good overall resolution was obtained for all compounds including their positional isomers in less than eight minutes using optimized UHPSFC conditions. All of the controlled "bath salts" could be distinguished by a combination of retention time and MS detection.

Lastly, UHPSFC separations for the above emerging drugs were compared to separations obtained by both GC and Ultra High-Pressure Liquid Chromatography (UHPLC), with particular attention paid to the separation of positional isomers and diastereomers. The latter techniques can be particularly problematic for the separation of positional isomers.[1] The degree of orthogonality of UHPSFC, GC, and UHPLC was demonstrated for the separation of both synthetic cannabinoids and bath salts using principal component analysis.

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Reference(s):

1. Marginean I., Rowe W.F., Lurie I.S. The role of ultra high performance liquid chromatography with time of flight detection of the identification of synthetic cannabinoids in seized drugs, *Forensic Sci. Int*. 249 (2015) 83-91.

Synthetic Cannabinoids, Synthetic Cathinones, Supercritical Fluid

B128 Identification of Regioisomers Via Gas Chromatography Coupled With Vapor-Phase Infrared Detection (GC-IRD)

Janice L. Aleman, BS, 510 Deerview Drive, Fredericksburg, TX 78624; Jesse M. Zavala, MS, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; Kyle E. Vircks, MS, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; and Warren C. Samms, PhD, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will be informed about the utility of using GC-IRD to differentiate regioisomers of abused substances.

This presentation will impact the forensic science community by providing information about how to utilize GC-IRD to distinguish regioisomers that are often difficult to differentiate using common methodologies. Due to identical mass spectra and similar retention times, laboratories are often unable to distinguish regioisomeric groups. The ability to differentiate these isomers allows the forensic science community to unambiguously identify newly designed substances. This is especially important when one isomer is controlled but another is not.

Designer drugs are appearing at an alarming rate across the world, requiring forensic chemists to develop new techniques for analysis. In many jurisdictions, designer drugs circumvent existing laws and are sold online or in smoke shops. In a previous validation study, various isomer sets of designer phenethylamines and cathinones were investigated. During the validation process, a few compounds presented potential data quality issues, such as low reproducibility of IR spectra resulting in incorrect library matches. Four of these compounds were 2,3-dimethylmethcathinone (2,3-DMMC), 3,4-dimethylethcathinone (3,4-DMEC), 2-methylmethcathinone (2-MMC), and 3-methylmethcathinone (3-MMC). Interestingly, the standards 2,4-dimethylmethcathinone (2,4-DMMC) and 2,4-dimethylethcathinone (2,4-DMEC) did not present these issues during the initial study and data was satisfactorily acquired. In the present study, analytical standards of 2,3-DMMC, 3,4-DMEC, 2-MMC, and 3-MMC were studied along with 5-(2-aminopropyl) indole (5-IT), alpha-methyltryptamine (AMT), as well as others. Prior to this investigation, this drug chemistry laboratory was unable to confirm these substances because they could not be separately and unambiguously identified. This is problematic in Texas, and presumably other states, as AMT is a controlled substance, while 5-IT is not. The additional compounds studied included controlled and non-controlled substances under Texas law.

To determine if the spectral details were stable and reproducible over time with the goal of capturing the optimal spectrum for library entry, standards for each substance were analyzed via GC-IRD twice daily for five days. Visual as well as library match analysis of each standard's chromatography and vapor phase IR spectra was conducted. Spectra were examined for their reproducibility over time, as well as any visually distinguishing features between closely related isomer sets.

A significant difference was noted between 5-IT and AMT vapor phase IR spectra and chromatography. IR spectra for both standards were found to be highly reproducible over the course of this study. Differences observed in the IR spectra were the sharpness and general peak shape primarily within the fingerprint region. AMT reproducibly suffered poor chromatography whereas 5-IT chromatography was consistently ideal.

The four cathinone controls, 2,3-DMMC, 3,4-DMEC, 3-MMC, and 2-MMC constituted in methanol, initially presented issues with chromatography. Therefore, a basic (0.45 N sodium hydroxide)/hexane extraction was performed and resulted in improved chromatography. It was noted that two of the four cathinone controls (2,3-DMMC and 2-MMC) began to degrade at a faster rate when compared to others during the validation process, resulting in low reproducibility for their IR spectra and lower likelihood of identification via library search. Frequent manipulation of averaged peak areas and background reference areas was required to compensate for decomposition products. Degradation of the injected samples could possibly be due to the instability of the compound itself, method parameters, or interaction of the sample with the reflective coating of the light pipe. Method optimization was attempted by changing a variety of parameters such as split ratios, flow rates, oven programming, and inlet temperatures. Overall, differentiation of these compounds was successful using obtained IR spectra.

Following this study, the analysis of forensic casework containing these substances resulted in unambiguous identification, allowing the laboratory to confirm which isomer was present in a given sample. These cases, along with other factors relating to positive identification via GC-IRD, will be discussed.

Drug Analysis, Regioisomers, GC-IRD

B129 Capillary Electrophoresis/Mass Spectrometry (CE/MS) as an Effective Tool for Identification of Illicit Drugs and Their Optical Isomers

Mehdi Moini, PhD, George Washington University, Dept of Forensic Sciences, 2100 Foxhall Road, NW, Washington, DC 20007; Christopher M. Rollman, BS, George Washington University, 2100 Foxhall Road, NW, Somers Hall, L14, Washington, DC 20007; Mike Hitchcock, MS, U.S. Postal Inspection Service, Forensic Laboratory Services, 22433 Randolph Drive, Dulles, VA 20104-1000; and Ioan Marginean, PhD, George Washington University, 2100 Foxhall Road, NW, Somers Hall L14C, Washington, DC 20007*

After attending this presentation, attendees will better understand the application of CE/MS for the separation of chiral compounds of forensic importance.

This presentation will impact the forensic science community by suggesting a rapid and highly sensitive method to separate and identify drugs and their optical isomers.

Many pharmaceutical and illicit drugs have structural and optical isomers. For some illicit drugs, identification of these is essential since scheduling and sentencing could vary based on which isomer of a compound is present in a sample. For example, amphetamine and Lyric® (S-pregabalin) are drugs which may be legally available as a single optical isomer. Also, information about the route of synthesis and country of production can be gained from isomer analysis. Amphetamines and cathinones can be synthesized through several routes which may result in different isomer quantities in the final drug. While Gas Chromatography/Mass Spectrometry (GC/MS), High-Performance Liquid Chromatography (HPLC), and Ultra High-Performance Liquid Chromatography/Mass Spectrometry (UHPLC/MS) are capable of separating some structural isomers, they require chiral derivatization or chiral columns for separation of optical isomers; however, these procedures are time-consuming and/or expensive. Capillary Electrophoresis/Ultraviolet (CE/UV) and High-Performance Liquid Chromatography/Ultraviolet (HPLC/UV) are also used for structural and optical isomer identification, but they lack confirmatory compound identification capability. Therefore, the forensic community will benefit from a confirmatory technique that can provide structural and optical isomer identification with ease. This presentation introduces a simple CE/MS technique for identification of structural and optical isomers of illicit drugs using (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (18-C-6-TCA) and Cyclodextrin (CD) derivatives as an effective background electrolyte that can provide optimum separation and high sensitivity detection of chiral compounds.

Analyses were performed using narrow (<20µm inner diameter), underivatized fused silica capillaries utilizing a porous tip for CE/MS interface. Various chiral selectors such as 18-C-6-TCA and CD derivatives were used as background electrolytes for CE/MS analyses. Samples were injected hydrodynamically and separations occurred at 25kV.

The separation of R- and S-pregabalin was achieved using only 18-C-6-TCA in approximately ten minutes. Quantitative studies showed a linear trend across three orders of magnitude between 1µg/mL and 1,000µg/mL. Furthermore, analysis of a Lyrica® tablet resulted in only one electrophoretic peak confirming the compound optical purity. In addition to pregabalin, the optical isomers of cathinone and nor-mephedrone (metabolite of mephedrone) formed complexes with 18-C-6-TCA, which allowed for their separation. The optical isomers of amphetamine and methamphetamine were separated using highly sulfated γ-CD. Future work includes analyzing more drugs whose chirality is important either due to scheduling or for intelligence purposes.

Using a background electrolyte containing 18-C-6-TCA or CD, CE/MS allows for the separation and high sensitivity detection of isomers.

Illicit Drugs, Optical Isomers, CE/MS

B130 Characterization of Synthetic Phenethylamines Using High Resolution Mass Spectrometry (HRMS)

Alexandria Anstett, BS, 2900 Northwind Drive, Apt 726, East Lansing, MI 48192; Fanny Chu, BS, 35-11 Linden Place, Apt 3F, Flushing, NY 11354; and Ruth Waddell Smith, PhD, Michigan State University, School of Criminal Justice, 560 Baker Hall, East Lansing, MI 48824*

After attending this presentation, attendees will understand the advantages of HRMS for the analysis of synthetic designer drugs, specifically synthetic phenethylamines. Attendees will learn how the mass defect obtained via HRMS can be used to characterize these compounds according to structural class.

This presentation will impact the forensic science community by providing a method to characterize unknown synthetic designer drugs according to structural class. This method is especially useful in the characterization of novel designer drug analogs for which no reference standard is yet available.

A number of different classes of synthetic designer drugs are known, including synthetic cannabinoids, phenethylamines, and cathinones. Compounds within each class often share a core structure and differ only in the position or identity of substituents. When a compound is regulated, a new analog often appears on the market. This analog exhibits similar psychoactive effects but because the structure is slightly different, the analog may not regulated under current legislation.

Samples submitted to the laboratory that may or may not contain controlled substances are analyzed by Gas Chromatography/Mass Spectrometry (GC/MS). This technique satisfies the Scientific Working Group for the Analysis of Seized Drugs recommendations, allowing definitive identification of controlled substances through a comparison of the mass spectrum obtained for the submitted sample to that of a reference standard. The GC/MS instruments used are typically configured with Electron Ionization (EI) and a single quadrupole mass analyzer (qMS). Electron ionization results in substantial fragmentation and the fragment ions observed can be used toward structural elucidation to identify the compound. The single qMS gives unit resolution, resulting in nominal mass data which is sufficient for identification of most controlled substances; however, synthetic designer drugs pose challenges that make definitive identification using GC/qMS difficult. First, isomers have the same nominal mass, making distinction by GC/qMS alone problematic. Second, because novel designer drug analogs appear on the market frequently, reference standards are often not immediately available, making identification challenging.

The objective in this research was to address the above issues by investigating the applicability of HRMS as a tool to aid in the identification and characterization of synthetic phenethylamines. Using HRMS, the accurate mass of each fragment ion is measured, providing additional understanding about the fragmentation pathway of the compound. This knowledge of the fragmentation behavior can be used to determine the compound structure with a high degree of certainty and therefore distinguish isomers. Further, accurate mass data is used to determine the mass defect of a compound, defined as the difference between the accurate and nominal mass. Compounds with similar core structure have similar mass defects. Thus, the mass defect can be used to determine the structural class of a new analog, which is useful in cases where no reference standard exists.

In this research, a set of synthetic phenethylamines from different subclasses (e.g., 2C and NBOMe) was investigated. Reference standards were prepared in methanol (1mg/mL) and analyzed in replicate by GC/qMS and by GC/Time-of-Flight (TOF) MS. Both instruments use EI; however, the TOF is a high-resolution mass analyzer, affording accurate mass data. For each standard, GC/qMS and GC/TOF/MS data were compared and showed similar fragmentation patterns. For example, characteristic fragment ions for 2C-E were observed at m/z 209Da, 180Da, and 165Da via GC/qMS and at m/z 209.1457Da, 180.1185Da, and 165.0947Da via GC/TOF/MS. The accurate mass data were used to confirm fragmentation pathways of the phenethylamines from which distinction of isomers such as 2C-E and 2C-G was possible.

For each standard within a subclass, the mass defects of the molecular ion and characteristic fragment ions were determined. From this, a range of mass defects representative of the core structure was calculated. For example, a mass defect range for the 2C subclass of 133.88±26.02mDa was determined based on the molecular ion for all 2C phenethylamines in the data set. An additional set of phenethylamine standards was used to evaluate the efficacy of the mass defect range for characterization. Successful classification was achieved despite the presence of some false positives and false negatives.

This presentation demonstrates the utility of HRMS in the identification and characterization of synthetic phenethylamines, focusing on the potential of mass defect as a characterization tool.

Synthetic Designer Drugs, Mass Spectrometry, Mass Defect

B131 Differentiation of Cathinone Isomers Using High Resolution Collision-Induced Dissociation Mass Spectrometry (CID/MS)

Cynthia Kaeser, MS, 162 Martin Avenue, Livermore, CA 94551; A. Daniel Jones, PhD, 219 Biochemistry, East Lansing, MI 48824; and Ruth Waddell Smith, PhD, Michigan State University, School of Criminal Justice, 560 Baker Hall, East Lansing, MI 48824*

After attending this presentation, attendees will understand how CID/MS fragmentation of cathinones can be used to differentiate isomers and how the underlying fragmentation mechanisms lead to the observed differences.

This presentation will impact the forensic science community by demonstrating the differentiating power of CID/MS for isomer identification. This presentation will provide better understanding of fragmentation mechanisms that can also be applied to analogs when no standard is available for comparison.

With the increased availability and subsequent regulation of synthetic designer drugs such as cathinones, clandestine chemists continue to produce new unregulated analogs of these compounds. These analogs keep many of the same structural features that yield the desired biological response but contain slight structural differences to avoid regulation. Isomers are especially difficult to distinguish because these analogs have the same chemical formula but with different structural arrangements. Typical analysis of a suspected cathinone by a forensic laboratory uses Gas Chromatography/Mass Spectrometry (GC/MS) with the Electron Ionization (EI) MS spectra compared to reference standards.

However, positive identification of new analogs by GC/MS is complicated by the lack of molecular ion and available reference standards. In these cases, several analytical techniques are necessary for identification, a difficult requirement to meet given the high caseloads and lack of advanced instrumentation available in many laboratories. As an alternative to using multiple instruments, CID/MS can be performed with high-mass resolution while using multiple collision energies for fragmentation. The main advantages of this technique over EI/MS are twofold. First, varying the collision energy used produces both the intact ion and a series of increasingly smaller fragments as the energy is increased. This reveals more details about the structural arrangement of an isomer than using a single collision energy. Second, high-mass resolution improves confidence in molecular formula assignment for positive identification.

While part of a larger study of cathinone fragmentation, three cathinone isomers were selected for this presentation: butylone, ethylone, and the 2,3-ethylone isomer. Butylone and ethylone are in Schedule I of the Controlled Substances Act while the 2,3-ethylone isomer is currently unregulated. Butylone and ethylone differ in the length of hydrocarbon chains at the alpha position and on the amine while the 3,4-methylenedioxy substitution on the aromatic ring is the same for both. The 2,3-ethylone isomer has the same hydrocarbon arrangement as ethylone, but the methylenedioxy group is at the 2,3-position.

Each standard was prepared at a concentration of 5ug/mL in methanol and directly injected into a quadrupole time-of-flight mass analyzer with Electrospray Ionization (ESI) and a leucine-enkephalin reference compound to improve mass accuracy. Fragmentation of each isomer was observed using collision energies of 10eV, 20eV, and 40eV. Resulting mass spectra were interrogated and the molecular formulas of major fragments were determined using their exact masses (four decimal places, <20ppm mass error). The molecular formulas and the voltage of appearance for each isomer's fragments were used to generate flowcharts for identification of common fragmentation pathways. These pathways provide insight into the positions of functional groups on the molecule allowing isomers to be distinguished.

While some differences in fragmentation were observed at the lowest collision energy, more discriminating features were observed at the higher energies. For example, at 10eV the loss of the amine group from the intact molecule distinguished butylone from the ethylone isomers. The fragment masses alone could not distinguish between the two ethylone isomers, although some differences in the relative abundances of each fragment were present. At higher voltages, additional fragments were identified by their exact masses to include losses of both odd and even electron species. While radical (odd electron) losses are less common for ESI than for EI, such species are resonantly stabilized by the aromatic ring. Radical fragments, including losses of radical methyl and ethyl groups, were the most differentiating between the two ethylone isomers.

This presentation will demonstrate the use of CID/MS as a single technique for successful isomer differentiation of cathinones. While this presentation focuses on a limited subset of cathinones, the process for using the observed fragmentation mechanisms for the identification of new cathinone analogs will also be discussed.

Cathinones, Mass Spectrometry, Designer Drugs

B132 The Effects of Water Immersion on the Recovery of DNA From Human Remains

Ema H. Graham, University of New Haven, 300 Boston Post Road, West Haven, CT 06516; Shanae J. Armstrong, MS, 2432 Oceancrest Boulevard, Far Rockaway, NY 11691-1929; and Michael S. Adamowicz, PhD, University of New Haven, Dept of Forensic Science, 300 Boston Post Road, West Haven, CT 06516*

After attending this presentation, attendees will better understand the effects of short duration (≤1 month) immersion in different types of water on human remains. This study presents data regarding the quantity and quality of DNA recovered from human soft tissue and bone after immersion in fresh, brackish, and salt waters. Data is shown for immersion periods ranging from 24 hours to 1 month in each type of water.

This presentation will impact the forensic science community by demonstrating that the quantity and quality of DNA recovered from different tissues is affected by both the duration of immersion and by the type of water in which the remains are immersed. This data will allow forensic scientists to better predict the success of DNA typing from human remains based on estimated time of immersion and water type, thus assisting in producing the most complete Short Tandem Repeat (STR) profiles possible.

There are numerous incidents that involve human remains in water and, as a result, identification of the individuals from these remains is of great importance. The purpose of this study was to determine if a DNA profile could be retrieved from human bone and soft tissue immersed for varying periods of time. It also assessed the effects of different water types on recovering usable DNA from the tissue samples. Human rib and associated muscle/connective tissue samples were collected from multiple individuals during autopsy. The soft tissue was separated from the bones, which were then equally sectioned, and both were immersed in salt water (~35ppt), brackish water (~10ppt), or fresh water (~0ppt) for intervals of 24 hours, 48 hours, 72 hours, 96 hours, 1 week, and 1 month (31 days). Time-control samples were also used for each of the different intervals, with no immersion in water. A time zero sample for each tissue type was run for each experiment to ascertain the approximate starting quantity and quality of the DNA.

DNA from the bone and soft tissue samples was extracted using QIAamp®, quantified with Quantifiler®, amplified via PowerPlex® 16 HS, and analyzed using standard methods of STR analysis employed in forensic laboratories. The DNA profiles generated were compared to the reference samples to determine how many of the alleles present were concordant with the references. DNA quality in each sample was assessed by averaging the number of heterozygous loci with peak height balances that were ≥70% and by comparing the average peak heights at loci D3S1358 and FGA for each immersion time interval.

Resulting data established that DNA degradation increased as time increased. All samples yielded full STR profiles after 24 hours of immersion with no significant DNA degradation present. After 48 hours of immersion, some samples, particularly bone in salt and brackish water, began to show allele and/or locus drop-out and heterozygous peak height imbalance indicating DNA degradation; however, full profiles were not uncommon at 48 hours. There was a significant reduction in the DNA quantity and quality in all sample types between the 48-hour and 72-hour immersion times. After 72 or more hours of immersion, some samples yielded full profiles, but locus drop-out and heterozygous peak height imbalance were common. While DNA degradation was severe after one month of immersion, typable STR results could still be produced in some samples. The results for soft tissue samples showed that after one month of immersion, samples in salt water had the highest DNA loss but produced the best quality DNA as measured by heterozygous peak height balance as compared to the same immersion time in fresh or brackish waters. After one month of immersion, bone samples in fresh water had the lowest DNA loss and produced the best quality DNA as compared to the same immersion time in salt or brackish waters.

DNA, Human Remains, Water Immersion

B133 Comparison of Three Filtration Devices for Recovery of Low Level and Degraded DNA

Nichole M. Tuscher, MFS, Contra Costa County Criminalistics Laboratory, 2530 Arnold Drive, Martinez, CA 94553; Ismail M. Sebetan, MD, PhD*, National University, Forensic Sciences Program, 11255 N Torrey Pines Road, La Jolla, CA 92037-1011; and Paul Stein, PhD*, National University, Forensic Science Program, 11255 N Torrey Pines Road, La Jolla, CA 92037*

After attending this presentation, attendees will understand the use of filter devices available for DNA recovery and which device may produce a better DNA yield for low level and compromised samples and will assist the DNA analyst in making decisions with respect to delicate forensic evidence. This presentation is based on an in-house comparison of the Microcon® 30 MW, Vivacon® 30 MW, and the NucleoSpin® devices for DNA capture using a phenol-chloroform extraction method.

This presentation will impact the forensic science community by demonstrating that an appropriate filter device can impact the outcome of DNA recovered for low level and compromised samples.

There is a strong demand in forensic DNA to analyze smaller and smaller amounts of DNA in casework. Many of these cases may involve "touch" DNA that may have inhibitors present. While phenol extraction is more labor intensive than robotic extraction methods, it permits the analyst to use a filter device to reduce the volume of the DNA extract into the small amplification volume required and increase the chances of template DNA recovery.

The Microcon®, Vivacon®, and NucleoSpin ® products all use a membrane-based filtration device for DNA capture. The Microcon® device can hold up to 500µl of volume at a time, the NucleoSpin® can accommodate up to 400µL of volume, and the Vivacon® can hold up to 2mL. All three devices use centrifugal force to filter the volume of the extract and washing buffer through the membrane. The Microcon® and Vivacon® require inversion of the membrane and centrifugal force to release the DNA in the elution buffer. The NucleoSpin® uses a binding buffer which permits washing of the membrane to remove inhibitors and then an elution buffer to release the DNA. The centrifugal time required to spin the buffers and wash is significantly shorter than the Microcon® or Vivacon®.

A series of experiments was set up in a side-by-side comparison of all three devices. An Applied Biosystems® 7500 Real-Time Polymerase Chain Reaction (RT-PCR) instrument with the Applied Biosystems® Quantifiler® Duo Kit was used to estimate the amount of DNA recovered. Only the inhibited and degraded samples were amplified with the Applied Biosystems® Identifiler® Kit and analyzed with an Applied Biosystems® 310 Genetic Analyzer. When comparing known amounts of low level DNA, the Nucleospin® had nearly twice the DNA recovery (91.5pg) compared to the Microcon® (48.6pg), and Vivacon® (50.8pg) at the lowest known concentration of DNA (111.2pg). As the concentrations increased, the Microcon® was comparable and then exceeded the NucleoSpin® at the lowest concentration 111.2pg of known DNA.

Inhibition was studied using different concentrations of soil and denim dye solutions added to the samples after digestion and before extraction since these are commonly encountered inhibitors. The Microcon® and Vivacon® were not able to produce any allele calls whereas the NucleoSpin® produced full profiles at all concentration levels. A degraded blood stain was diluted to create known low level samples to test the recovery ability of the Nucleospin® and Microcon®. The NucleoSpin® generated partial profiles with three different dilutions and the Microcon® produced one partial profile for one dilution and a few allele calls with the remaining dilutions.

In conclusion, the NucleoSpin® was not only more effective at removing inhibitors as anticipated by the literature but was also better at recovering degraded DNA than the Microcon®. The added benefit of the reduced amount of sample handling and shorter centrifuge time makes the NucleoSpin® product a better concentration device for low level and compromised DNA samples.

Low Level DNA, PCR Inhibitors, DNA Filtration Device

B134 Selective Degradation Using the Erase™ Sperm Isolation Kit and PrepFiler® Purification

Melissa D. Moore, BS, Orange County Crime Lab, 320 N Flower Street, Santa Ana, CA 92703; Richard A. Gustilo, Orange County Crime Laboratory, 320 N Flower Street, Santa Ana, CA, CA 92703; Mary M. Hong, BS, Orange County Sheriff-Coroner, 320 N Flower Street, Santa Ana, CA 92703; Ruth H. Ikeda, PhD, Orange County Sheriff-Coroner, 320 N Flower Street, Santa Ana, CA 92703; Stacy Vallercamp, 300 N Flower Street, Santa Ana, CA 92703; Ismail M. Sebetan, MD, PhD*, National University, Forensic Sciences Program, 11255 N Torrey Pines Road, La Jolla, CA 92037-1011; and Paul Stein, PhD*, National University, Forensic Science Program, 11255 N Torrey Pines Road, La Jolla, CA 92037*

After attending this presentation, attendees will understand the benefits of using selective degradation in place of the standard differential extraction method when processing sexual assault evidence. Attendees will learn how selective degradation can be used to replace the standard differential extraction method to obtain a single-source male DNA profile and how the process can be partially automated by integrating the Erase™ Sperm Isolation Kit with the PrepFiler® DNA Extraction Kit.

This presentation will impact the forensic science community by introducing a reliable procedure that can be successfully used for differential extraction of DNA from very challenging forensic samples in a shorter time and with less intensive labor.

This presentation discusses how selective degradation can be used to replace the standard differential extraction method to obtain a single-source male DNA profile from post-coital vaginal swabs containing sperm. Differential extraction is traditionally used to separate and purify the sperm cell DNA from the epithelial cell DNA. Differential extraction is time consuming and requires intensive work by the analyst. With the high number of sexual assault cases and increasing backlog of sexual assault kits, it is necessary to implement a simpler method to separate sperm cell DNA from epithelial cell DNA.

Selective degradation is accomplished by selectively destroying epithelial DNA using a nuclease, while sperm DNA remains intact inside the sperm cell. The Erase™ Sperm Isolation kit provides crime laboratories with the components necessary to perform selective degradation on sexual assault evidence.

Once the epithelial cell DNA and sperm cell DNA are separated using selective degradation, the DNA sample must be purified. The Erase™ protocol states that the DNA sample can be purified using ethanol precipitation, size filtration, or QIAGEN® EZ1 DNA purification. This study determined that the PrepFiler® DNA Extraction kit can also be used to purify DNA samples previously digested with the Erase™ Sperm Isolation kit. Although the selective degradation portion of this method is performed manually, the DNA purification portion of this method can be performed automatically using the Tecan Freedom EVO® 150, which is a robotic liquid handler. The usage of a robotic liquid handler greatly reduces the amount of analyst hands-on time, variability, and exposure to harmful chemicals, such as phenol-chloroform. Once purified, the DNA sample can be used in further downstream applications.

Vaginal swabs were first collected zero to one hour after intercourse and for the purpose of this study are considered high-sperm samples. Vaginal swabs were collected again between 21 and 25 hours after intercourse and considered low-sperm samples. Stained microscope slides were prepared to identify the presence of spermatozoa. Microscopic identification of spermatozoa confirmed that each of the four samples tested contained a low amount of sperm.

The low-sperm post-coital swabs collected at 21 to 25 hours were digested using the Erase™ Sperm Isolation kit protocol and purified using the automated PrepFiler® DNA Extraction kit. After purification, the sperm and non-sperm fractions of the DNA sample were quantitated, amplified, and typed using Identifiler Plus®. The sperm fraction DNA profiles resulted in single-source male DNA profiles 87.5% of the time. For the purposes of this study, a DNA profile was considered single-source when all loci were identified as originating from one individual and where the minor peak represented was less than 20% at each locus. If the sperm fraction DNA profile had female signals between 20% and 80% of the total signal at a locus, it was considered a mixed profile.

Experimental findings show that the Erase™ Sperm Isolation kit can successfully separate epithelial cell DNA from sperm cell DNA located on post-coital vaginal swabs containing a low amount of sperm. Results indicate that the Erase™ Sperm Isolation kit can be integrated with the PrepFiler® DNA Extraction kit to separate and purify epithelial cell DNA from sperm cell DNA.

This study shows that selective degradation combined with automated DNA purification can be used in place of the standard differential extraction method on sexual assault evidence containing a mixture of DNA from the victim, in the form of epithelial cells, and from the perpetrator, in the form of spermatozoa. Using selective degradation as opposed to the standard differential extraction method when processing sexual assault evidence can conserve time and effort and result in better sperm fraction DNA profiles.

Differential Extraction, Selective Degradation, Sexual Assault Evidence

B135 Evaluation of the QIAGEN® Investigator 24Plex Polymerase Chain Reaction (PCR) Kit for Amplification of Forensic Samples

Clinton D. Buchanan, PhD, 4930 N 31st Street, Forest Park, GA 30297; and Joel D. Sutton, MSFS, 4930 N 31st Street, Forest Park, GA 30297-5205*

The goal of this presentation is to provide attendees with information regarding performance of the QIAGEN® Investigator 24plex PCR kit during assessment studies addressing sensitivity, reproducibility, precision, mixtures, contamination, concordance, and non-probative case-type samples.

This presentation will impact the forensic science community by providing insight to laboratories considering implementation of expanded core loci PCR kits for forensic casework applications.

The QIAGEN® Investigator 24plex QS kit is a recently National DNA Index System (NDIS) -approved Short Tandem Repeat (STR) amplification kit used for human identification applications. The kit allows for multiplex amplification of the Combined DNA Index System (CODIS) core loci, the European Standard Set (ESS) markers, SE33, DYS391, D2S1338, D19S433 and Amelogenin. Laboratory studies of the QIAGEN® Investigator 24plex QS were performed to determine the efficacy of the kit for amplification of forensic samples. DNA samples were amplified using the standard cycling protocol, and sample amplicons were processed on the Applied Biosystems® 3500xl genetic analyzer following steps outlined in the Investigator 24plex QS handbook (rev. 08/2014). GeneMapper ID-X_v1.2 panels, bins, and stutter files for the Investigator 24plex QS kit were provided by QIAGEN® (Investigator_TemplateFiles_GeneMapper_ID-X_v7). These default files were used for data analysis; however, default stutter ratios for some loci (provided in the Investigator Template GeneMapper ID-X _v7 files) were modified based on maximum stutter findings observed.

The experiments focused on sensitivity and mixtures prepared from DNA sourced commercially and via in-house extraction methods. Comprehensive mixture studies were conducted. Additional work included reproducibility, concordance, contamination assessment, non-probative case-type samples, precision, and qualifying samples (National Institute of Standards and Technology (NIST) standard reference material) studies. Artifacts observed throughout the course of testing included elevated baseline, spectral pull-up, elevated stutter, and occasional spikes. DNA template amounts ranging from 0.008ng to 1.25ng from multiple sources were included in sensitivity studies. Admittedly, elevated stutter is not unexpected in lower-level DNA samples; however, stutter values exceeding filter values provided in the Investigator Template GeneMapper ID-X _v7 files were observed in samples associated with DNA template amounts ≥0.125ng. In addition, allelic drop-in of a 14 allele at locus D8S1179 was observed in one 9948 sensitivity sample at 0.016ng; however, this is not unexpected when working with lower-level DNA samples. Heterozygous peak height ratios were generally ≥50% in sensitivity samples targeting ≥0.125ng of DNA, and complete profiles were generally observed in samples targeting ≥0.063ng of DNA. The data gathered and analyzed from the laboratory studies of the QIAGEN® Investigator 24plex QS kit supports the PCR kit as being a robust and reliable tool for obtaining STR results for the expanded CODIS core loci set.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. Names of commercial manufacturers or products included are incidental only, and inclusion does not imply endorsement by the authors.

QIAGEN® Investigator 24-Plex, PCR Kit, Forensic Samples

B136 Working With Challenging Samples: An Independent Assessment of the Relative Performance of the Promega® Fusion™ and InnoGenomics® InnoTyper™ Kit With Probative Samples

James Anstead, PhD, 840 Research Parkway, Ste 551, Oklahoma City, OK 73104; Erica Reynaga, MS, DNASolutions, 840 Research Parkway, Ste 551, Oklahoma City, OK 73104; Kelsy Lowther, MS, DNASolutions, 840 Research Parkway, Ste 551, Oklahoma City, OK 73104; and Brandt G. Cassidy, PhD, 840 Research Parkway, Ste 551, Oklahoma City, OK 73104*

After attending this presentation, attendees will better understand the relative merits of the InnoGenomics® InnoTyper™ and Promega® Fusion™ kits for the analysis of challenging forensic samples.

This presentation will impact the forensic science community by providing information on gaining the most information from challenging forensic samples.

Traditional analysis of challenging forensic samples often relies on mitochondrial markers, due to their relative stability and high copy number; however, there are considerable drawbacks to this approach including the lack or recombination and the absence of paternal contribution. Therefore, the generation of reliable nuclear profiles from degraded samples continues to be increasingly important for forensic testing.

Recently, based in part on the recommendations from the Federal Bureau of Investigation (FBI), kit manufacturers have released multiplexes with heightened sensitivity and robustness when compared to standard genomic marker systems. These systems have been optimized for improved performance with challenging samples. The Promega® Fusion™ system interrogates 22 autosomal Short Tandem Repeats (STR), the amelogenin locus for gender identification, and a gender confirmatory marker on the Y chromosome. Eight of the STR markers produce alleles less than 200 base pairs in length, increasing the likelihood of producing results from compromised forensic samples and the chance of obtaining probative data from forensic evidence. The development of markers based on Retrotransposable Insertion Polymorphisms (RIPs) enables additional information to be retrieved from the most highly degraded and inhibited samples, including bone and hair shafts. Innogenomics® InnoTyper™ 21 system utilizes small amplicon DNA typing of repetitive Alu sequences from 20 loci and amelogenin in which each locus is scored for the presence of a stable heritable insertion. This biallelic system has been reported to produce interpretable genetic profiles with as little as 60 picograms of input DNA from compromised and challenging samples. This study assesses the sensitivity and amount of probative data obtained using the Promega® Fusion™ kit and the Innogenomics® InnoTyper™ 21 kit on low-yielding, highly degraded, and challenging forensic samples, including bone of varying age and condition, hair shafts, and touch samples.

InnoTyper™, Fusion™, STR

B137 Faux-Dis: An Online, Searchable DNA Database Available for Educational Purposes

Ashley Hall, PhD, University of Nebraska - Lincoln, 103 Entomology Hall, Lincoln, NE 68583*

After attending this presentation, attendees will better understand a novel, publicly available educational tool.

This presentation will impact the forensic science community by introducing a powerful research and teaching tool.

The United States national DNA database, Combined DNA Index System (CODIS), contains more than ten million genetic profiles originating from convicted offenders, missing persons, and crime scene evidence and is searchable only by authorized governmental agencies. Due to the private nature of the data it contains, educators do not have access to this powerful database for teaching purposes. Therefore, there is currently a lack of a searchable human DNA profile databases available for use in the research laboratory or in the classroom. If it were available, such a database could be a valuable tool in the analysis of large data sets, for use in forensic laboratory exercises, and in answering questions about population genetics. Therefore, the development of a searchable DNA database modeled after CODIS that can be used by educators nationwide was initiated. Called Faux-Dis, it will allow students to gain hands-on experience and knowledge in the manipulation of large data sets, and it would support calculations in population genetics problems. The development of this database is a beneficial contribution to the field of forensic science because it has the potential to foster a nationwide collaboration and can be used for graduate and undergraduate education and training.

To initiate this project, human biological DNA samples containing buccal epithelial cells were collected from donors using a sterile cotton swab. DNA was isolated and purified using an organic extraction. Each sample was then quantified using the Qubit® fluorometer. The multiplex Polymerase Chain Reaction (PCR) PowerPlex® 16 was used to amplify the DNA at 16 loci, which were targeted by specific, fluorescently labeled primers. The PCR products were run through capillary electrophoresis on the 3130 Genetic Analyzer to generate a DNA profile. This profile was visualized as a plot of Relative Fluorescent Units (RFU) against size in base pairs (bp) for each of the 16 regions (loci) amplified by PCR. Each locus has two alleles, giving either one or two peaks. The size in bp determines which alleles are present. Each allele can be determined by comparing to an allelic ladder, thus allowing the profile to be genotyped. The generated profiles were entered into the database.

To date, a standard workflow for processing samples has been established and 100 profiles have been added to the database. In the future, the database will be transferred to an online searchable format, and collaborators from other universities and colleges will be invited to submit samples for analysis to form a large consortium of participants.

CODIS, Database, Education

B138 Human Short Tandem Repeat (STR) Profiles From Blood-Fed Mosquitos

Jared Latiolais, MSc, 10430 Furnace Road, Lorton, VA 22079; Dane T. Plaza, BS, 10430 Furnace Road, Ste 107, Lorton, VA 22079; Andrew B. Feldman, PhD, 11100 Johns Hopkins Road, Laurel, MD 20723-6099; Mobolaje Okulate, PhD, University of Maryland Eastern Shore, 30665 Student Services Center Lane, Princess Anne, MD 21853; Nirbhay Kumar, Louisiana State University, Baton Rouge, LA 70803; and Robert A. Bever, PhD, Mitotyping Technologies, 2565 Park Center Boulevard, Ste 200, State College, PA 16801*

WITHDRAWN

B139 Examining the Contribution of Sampling to Peak Height Imbalance in Low Template DNA Samples Using a Single-Tube Extraction Protocol

Thutrang Nguyen, BA, 221 Massachusetts, #708, Boston, MA 02115; and Robin W. Cotton, PhD, Boston University School of Medicine, Biomedical Forensic Sciences, 72 E Concord Street, R 806, Boston, MA 02118*

After attending this presentation, attendees will understand that both pre-Polymerase Chain Reaction (PCR) sampling and PCR chemistry contribute to peak height imbalance as shown experimentally using a single-tube extraction and direct amplification protocol.

This presentation will impact the forensic science community by experimentally showing the contribution of pre-PCR sampling to peak height imbalance.

The developments of the PCR and the Short Tandem Repeat (STR) multiplex kits increased the ease and lowered the time and sample quantity required for Deoxyribonucleic Acid (DNA) typing compared to previous methods; however, the amplification of such a low mass of DNA can lead to increased stochastic effects, such as Allele Drop-Out (ADO) and heterozygous Peak Height (PH) imbalance, which make it difficult to determine the true donor profile. These stochastic effects are believed to be due to: (1) pre-PCR sampling from pipetting and sample transferal of dilute samples prior to amplification resulting in imbalanced heterozygous allele templates in the amplification reaction; and, (2) the kinetics of the PCR process in which there may be uneven amplification of heterozygous alleles during early PCR cycles when few target templates are initially available.

This study examines the contribution of PCR chemistry and pre-PCR sampling errors on stochastic effects by utilizing a single-tube DNA extraction and direct amplification method. Cells were collected into tubes using the McCrone Associates, Inc. cell transfer method, which allowed for approximation of DNA mass without quantification. The forensicGEM® saliva kit was used to lyse the cells and inactivate nucleases without inhibiting downstream amplification. The samples were then directly amplified with the AmpFℓSTR® Identifiler® Plus PCR Amplification kit. These samples should only show the effects of PCR chemistry since pipetting and tube transferal steps prior to amplification were removed with the expectation that equal numbers of heterozygous alleles are present in the sample pre-amplification. Comparisons of PH imbalance were made to samples extracted with forensicGEM® but had one or more pipetting and tube transferal steps prior to amplification; thus, these samples would exhibit the effects of both pre-PCR sampling and PCR chemistry errors and inefficiencies. Results indicate the imperfections observed in PH ratios are a combination of sampling and PCR-generated stochastic effects.

Peak Height Ratio, Low Template DNA, Single-Tube Extraction

B140 Working to Solve Compatibility Issues Between Impression Enhancement and DNA Analysis

Jessica Zarate, MS, 36600 Schoolcraft Road, Livonia, MI 48150; and Jodi Lynn Barta, PhD, Madonna University, 36600 Schoolcraft Road, Livonia, MI 48150*

After attending this presentation, attendees will be provided with research that explores the need for collaborative efforts to maximize evidence potential in order to provide both impression details and DNA profiles for evidence samples deposited in biological fluids. Highlighted in this presentation are the data generated during National Institute of Justice (NIJ) -funded research that uses Zar-Pro™ fluorescent blood lifting strips for enhancement and preservation along with common DNA extraction techniques to generate DNA profiles from impression evidence that has been lifted.

This presentation will impact the forensic science community by showing how DNA is preserved and can be retrieved from impression evidence lifted and enhanced using Zar-Pro™ fluorescent blood lifting strips, thus simplifying collection and preservation while expanding the utility of impression enhancement methods including DNA analysis, which could have the potential to change the way technicians approach crime scene evidence.

Impression evidence, deposited in both blood and non-blood biological fluids, is a common component at many crime scenes. Current fluorogenic enhancement methods for impression evidence can be problematic for DNA preservation and are often impractical for crime scene use due to their toxicity. This may result in a situation in which a crime scene technician must make a decision during evidence collection either to enhance the impression (potentially damaging the DNA evidence) or to gather the DNA evidence (possibly destroying the impression evidence). Zar-Pro™ fluorescent blood lifting strips have been successful in lifting, enhancing, and preserving bloody impression evidence. They provide a highly sensitive method for processing and fluorogenically enhancing bloody impression evidence that can be preserved and utilized over long time intervals; however, the viability of subsequent DNA analyses has not been established.

This research explores the need for collaborative efforts to maximize evidence potential in order to provide both impression details and DNA profiles for evidence samples deposited in biological fluids. Highlighted in this presentation are the data generated during NIJ-funded research that uses Zar-Pro™ fluorescent blood lifting strips for enhancement and preservation along with common DNA extraction techniques to generate DNA profiles from impression evidence that has been lifted. During the initial phase of the project, more than 1,200 impressions were made in five biological fluids on a series of seven substrates that range from non-porous to semi-porous to porous. In order to determine the viability of the DNA over time when fixed to the Zar-Pro™ fluorescent blood lifting strips, trials were established to test one-month, three-month, six-month, and twelve-month intervals. The goal of this project was to test the viability of DNA in evidence processed with Zar-Pro™ fluorescent blood lifting strips and to develop and optimize a DNA extraction protocol in an effort to develop a simple, time- and cost-effective, non-toxic method that is safe for use at crime scenes and provides opportunities for subsequent DNA recovery suitable for use in forensic science laboratories. Preliminary results are encouraging and indicate that DNA is sufficiently preserved and can be retrieved from impression evidence lifted and enhanced using Zar-Pro™ fluorescent blood lifting strips. Research is ongoing; however, the data generated suggest that simplifying collection and preservation while expanding the utility of impression enhancement methods to include DNA analysis could change the way technicians approach crime scene evidence.

Impression Enhancement, DNA Preservation, DNA Analysis

B141 Updates to the Forensic Research/Reference on Genetics Knowledge Base (FROG-kb) Database

Kenneth Kidd, PhD, Yale University School of Medicine, Dept of Genetics, 333 Cedar Street, New Haven, CT 06520; Haseena Rajeevan, PhD, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520; Katherine N. Moore, MS, RTI International, 3040 E Cornwallis Road, Research Triangle Park, NC 27709; Richard Satcher, MS, RTI International, 3040 E Cornwallis Road, Research Triangle Park, NC 27709; Patricia A. Foley-Melton, PhD, RTI International Center for Forensic Science, 3040 E Cornwallis Road, Bldg 3, Rm 201, Research Triangle Park, NC 27709; and Jeri D. Ropero-Miller, PhD, RTI International, 3040 Cornwallis Road, PO Box 12194, Bldg 7, Rm 211, Research Triangle Park, NC 27709*

After attending this presentation, attendees will have an overview of the capabilities and functionality of the FROG-kb as a resource to make allele frequency data for Single Nucleotide Polymorphisms (SNPs) and other genetic polymorphisms more useful in the forensic field.

This presentation will impact the forensic science community by providing an overview of FROG-kb, recent updates to the database including data and website enhancements, and a visual of how to utilize FROG-kb.

FROG-kb (http://frog.med.yale.edu/FrogKB/) is a database that provides tools through its web interface for comparing user-provided data with underlying allele frequencies in populations and serves as a teaching and research web interface. Current SNP panels on FROG-kb include Individual Identification Single Nucleotide Polymorphisms (IISNPs), Ancestry Inference Single Nucleotide Polymorphisms (AISNPs), and Phenotype Interference Single Nucleotide Polymorphisms (PISNPs). The data used in FROG-kb calculations derive from the Allele Frequency Database (ALFRED) (http://alfred.med.yale.edu), a continually updated database of allele frequency data on SNPs and other genetic polymorphisms. SNPs can be valuable for investigative leads when known profiles using STRs are not available and no hits in databases such as the Combined DNA Index System (CODIS) are identified. These investigative leads provided by SNPs can include ancestry and phenotype inference and individual identification, all of which are provided in the FROG-kb database.

Recent updates to FROG-kb have centered on inputting additional data and website enhancements to provide an organized and easily navigated resource for the forensic community. One of these updates was creating a comprehensive user manual that provides users with step-by-step directions on how to use and search FROG-kb from the different SNP panels available. A short video was created to provide users a visual on navigating and searching FROG-kb along with search examples. This presentation will provide an overview of the new infrastructure to utilize the database for forensic purposes, including a user manual to assist in the process, discussion of the results of searches, and what can be done with the data.

Understanding and utilizing SNPs are important as a new area in the forensic field and FROG-kb serves to provide this to the forensic science community as a user-friendly, free, and web-accessible resource.

Allele Frequency, Database, FROG-kb

B142 Examination of 20 Retrotransposable Polymorphic Insertion/Null (INNUL) Markers for Their Utility in Kinship Testing Using the Commercial Software Program LSAM

AnniLauri Villeme, BS, 311 Eldrid Drive, Silver Spring, MD 20904; Gretchen E. Bartizal, 327 Montpelier Court, Westminster, MD 21157; Becky Hill, MS, 100 Bureau Drive, MS 8311, Gaithersburg, MD 20899; and Michael D. Coble, PhD, 100 Bureau Drive, MS 8312, Gaithersburg, MD 20899-8312*

After attending this presentation, attendees will understand the effectiveness and abilities of using the software program Laboratory Information System Applications (LISA) Statistical Analysis Module (LSAM) to successfully track and manage data and documents, calculate statistics of paternity/kinship analysis, and support direct match comparisons of profiles for the INNUL markers compared to hand-calculated statistics for paternity/kinship analysis in complete and degraded sample profiles.

This presentation will impact the forensic science community by illustrating the benefits and potential of using the LSAM software for the analysis of INNUL markers in kinship and paternity testing. Also, this study examines the impact that INNUL markers have compared to other types of markers such as mitochondrial DNA (mtDNA) or Single Nucleotide Polymorphism (SNP) when testing samples with degraded DNA.

Forensic DNA testing has proven to be a powerful tool for criminal investigations and for identifying human remains via kinship analysis for cases involving mass disasters or missing persons. Short Tandem Repeat (STR) markers most often offer the highest degree of discrimination and speed of analysis by using the Polymerase Chain Reaction (PCR) and capillary electrophoresis to generate DNA profiles. DNA testing for human remains identification is often challenging due to the presence of degraded DNA or inhibitors that affect the PCR reaction. Mini Short Tandem Repeats (miniSTRs) have been engineered to decrease the size of PCR amplicons to improve recovery of DNA fragments in the high molecular weight range of standard STR kits.[1] Additional marker systems such as SNPs, Insertion/Deletion markers (InDels), and mitochondrial DNA (mtDNA) have been successfully used for highly degraded samples.

Retrotransposable Polymorphisms (RP) are found in the human genome as RNA elements that have been reverse transcribed into specific loci as complementary DNA (cDNA) by use of retroposition.[2] RP include the Alu elements Short Interspersed Nuclear Elements (SINEs) and Long Interspersed Nuclear Elements (LINEs).[3] These elements can be in two allelic states: either they are present in an individual's DNA as an insertion or absent as a null (INNULs). One advantage for targeting these markers is the small amplicon size that can be created for each marker (approximately 60bp-125bp in size).[4] A commercially available kit, InnoTyper™ 21, containing 20 INNUL markers plus the sex determining marker, Amelogenin, is available and was used for this study.4

The allele frequencies and population genetic parameters of the markers in a set of more than 600 population samples at National Institute of Standards and Technology (NIST) were characterized first.[5] Samples with close relatives (father/mother/child trios) were then genotyped for testing. Next, individuals in the trios were artificially degraded using sonication to generate fragmented DNA. Samples were typed with the INNUL markers and a commercially available multiplex STR kit for determining the Random Match Probability (RMP).

The utility of the INNUL markers for paternity and kinship analysis were considered. The software package LSAM-LISA Statistical Analysis Module from Future Technologies Inc. was used for direct comparison statistics and to conduct pedigree statistics for the INNULs. The program offered the ability to construct pedigree scenarios for kinship analysis. The software statistics were compared to those determined by hand calculation.

It was found that the INNUL markers were able to provide additional genetic information for samples that were highly degraded. The LSAM software program provided strong support for the concordance to produce accurate calculations for kinship analysis compared to hand calculation.

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InnoTyper™, LSAM, INNUL

B143 Complex Mixtures and the Minimum Number of Contributors: A Case Study

Nathaniel D. Adams, BS, Wright State University, 3640 Colonel Glenn Highway, Dayton, OH 45435; Ranajit Chakraborty, PhD, University of N Texas Health Science Center, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107; Carrie Rowland, MSc, Wright State University, 2850 Presidential Drive, Ste 160, Fairborn, OH 45324; and Dan Krane, PhD, 3640 Colonel Glenn Highway, Dept Bio Sci, Dayton, OH 45435*

After attending this presentation, attendees will better understand the challenges associated with estimating the number of contributors in a complex DNA mixture and the importance of making accurate assessments before attempting probabilistic genotyping to ascertain likelihood ratios. Results from an empirical analysis of simulated mixtures using real genotypes will be described.

This presentation will impact the forensic science community by: (1) illustrating the difficulties of assessing the number of contributors to a mixed sample; (2) presenting an exploration of novel empirical mixture analyses; and, (3) showing the impact these analyses have had on recent investigations involving probabilistic genotyping.

The scenario considered for this simulation study consists of a case in which a forensic DNA testing laboratory developed genotypes from three injections of DNA extracted from a swabbing of the grip area of a firearm found to be associated with a crime. The testing laboratory performed probabilistic genotyping analyses on the multiple injections, resulting in a single Likelihood Ratio (LR) reported for the swab of the grip area.

The calculation of LRs in forensic DNA statistics requires an explicit assumption of a number of contributors in both the numerator and denominator. Despite the observation of seven unique peaks at a single locus, the testing laboratory generated LRs under the assumption that only three individuals contributed to the observed DNA profile, suggesting that the single observation of a seventh peak at one locus across three injections was an artifact rather than an indication of a real allele and a fourth contributor. The final reported LR supported the data under H1 (Hp; Defendant + two unknowns) as being 4,190 times more probable than under H2 (Hd; three unknowns). The probabilistic genotyping system used for these analyses has not been validated for analyzing samples containing DNA from more than three individuals (as of June 2015). As a result, the testing laboratory was unable to evaluate the alternative hypotheses that four or more individuals contributed to the mixture.

An empirical analysis of 361 Caucasian genotypes published by the National Institute of Standards and Technology (NIST) indicates that of the 15 genetic loci genotyped by the commercially available Identifiler® test kit, approximately half of simulated, known, four-person mixtures (N=695,946,630) would have no more than six unique alleles observed at any locus. When disregarding the single locus with the highest count of unique alleles (as is the practice of the testing laboratory for this case) approximately 90% of all four-person mixtures would have no more than six unique alleles observed across the remaining loci. The results of this analysis indicate that the use of maximum allele counts for assessing the number of contributors is frequently inaccurate, especially for three or more contributors, and that an abundance of caution should be exercised when evaluating LRs in such instances.

The testing laboratory suggested that it was "more cautious" to consider the evidence sample to be a mixture of at least three individuals (presuming that allelic drop-in was likely to have occurred) rather than a mixture of at least four without providing any LR or level of confidence to that assertion. When confronted with these results and their implications, the testing laboratory and prosecution chose to withdraw their conclusions regarding DNA testing in this case prior to a judge's ruling in an admissibility hearing in a federal court.

Complex Mixtures, Number of Contributors, Likelihood Ratios

B144 A Single Multiplex Polymerase Chain Reaction (PCR) Assay of Rapidly Mutating (RM) Y-Chromosomal Short Tandem Repeat (Y-STR) Loci to Complement Current Sets of Markers Used in Forensic Y-Chromosome Analysis

Daniela Lacerenza, PhD, University of Torino, Dept Public Health Sciences, Sezione di Medicina Legale, c.so Galileo Galilei #22, Torino 10126, ITALY; Giancarlo Di Vella, MD, PhD, University of Torino, Dept Public Health Sciences, Sezione di Medicina Legale, Corso Galileo Galilei 22, Torino 10126, ITALY; and Carlo Robino, 22, Torino, TO 10126, ITALY*

The goal of this presentation is to introduce a set of RM Y-STR markers that can complement commercially available multiplex kits.

This presentation will impact the forensic science community by highlighting the ability of additional RM Y-STR loci to resolve paternal lineages and discriminate closely related male suspects.

Male-specific Y-STR loci are especially useful in cases of sexual assault, where mixed stains that contain abundant DNA from the female victim together with trace DNA from the male assailant are analyzed. A general drawback of Y-STRs is that, compared with autosomal STRs, their ability to identify single individuals is limited. Since mutation is the only driving force of variation in the human Y chromosome, and currently used Y-STRs have low mutation rates (in the order of 1 x 10-3), groups of paternally related men, and in particular closely related males, cannot be easily differentiated. To improve the power of discrimination of conventional Y-STRs, it is therefore necessary to complement them with additional markers with much higher mutation rates. Through a systematic study, it was possible to identify a subset of 13 RM Y-STRs displaying a few mutations per marker every 100 generations.[1] Part of these markers have subsequently been included in commercial kits such as the PowerPlex® Y23 system by Promega® (DYS570, DYS576) and Y Filer® Plus by Life Technologies™ (DYS570, DYS576, DYS627, DYS518, DYS449, and DYF387S1).

In a forensic context, minimization of analytical steps is necessary to draw as much information as possible from minute amounts of DNA isolated from stains; however, amplification of the RM Y-STR markers (DYF399S1, DYF403S1ab, DYF404S1, DYS526ab, DYS547, DYS612, and DYS626) not already included in the Y Filer® Plus panel, which currently represents the most comprehensive commercial set available for both conventional and RM Y-STR loci, was originally described in three separate reactions.[2] The goal of this project was to combine these reactions in a single compact multiplex PCR assay.

PCR primers and amplification conditions for the seven RM Y-STRs included in the multiplex are those described by Robino et al., with the exception of a modification of the dye label for locus DYS626 (from 6-FAM to TAMRA) in order to avoid overlapping between marker-specific fluorescent signals.[3] Developmental validation of the assay included a sensitive study of serial dilutions of male control DNA (2800M) and a mixture study, in which the serial dilutions of male DNA were mixed 1:1 with a female control DNA sample (200ng/µl).

The sensitivity study showed that full RM Y-STR profiles were obtained from as little as 62.5pg of template male DNA, whereas partial profiles, including drop-out artefacts at the multi-copy markers DYF399S1, DYF403S1a, and DYF404S1, could be observed at lower concentrations. Results were not affected by the concurrent presence of female DNA. Though the Y Filer® Plus panel has been shown to greatly improve the resolution of paternal lineages, it does not achieve complete differentiation, especially in more isolated/endogamous populations.[4] The described multiplex can therefore complement Y Filer® Plus data in selected cases requiring further power of discrimination, in particular when closely related male suspects are involved. The main concern regarding the use of additional RM Y-STRs in trace DNA analysis is that the occurrence of allelic drop-out and drop-in must be considered in multi-copy markers with a non-fixed number of alleles.

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Sexual Assault, Y Chromosome, Rapidly Mutating Y-STRs

B145 Utility of InnoTyper™ 21 in Analysis of Degraded Human DNA Recovered From Maggot Crop Contents

Sharon E. Zeller, BS, 3940 Oakleys Lane, Richmond, VA 23223; Kyle S. Williams, Virginia Commonwealth University, 104 W Franklin Street, Apt 1703, Richmond, VA 23220; Sudhir K. Sinha, PhD, InnoGenomics Technologies, LLC, 1441 Canal Street, Ste 307, New Orleans, LA 70112; Gina M. Murphy, MS, GMP Forensic Consultants, PO Box 113006, Metairie, LA 70011-3006; Hiromi Brown, PhD, InnoGenomics, 1441 Canal Street, New Orleans, LA 70112; Daniel J. Wescott, PhD, Texas State University, Dept of Anthropology, 601 University Drive, San Marcos, TX 78666-4684; Tracey Dawson Cruz, PhD, Virginia Commonwealth University, 1015 Floyd Avenue, PO Box 843079, Richmond, VA 23284; and Baneshwar Singh, PhD*, Virginia Commonwealth University, Dept of Forensic Science, 1015 Floyd Avenue, Rm 2015, Richmond, VA 23284*

After attending this presentation, attendees will better understand the utility of a new human DNA typing method in nuclear DNA analyses of highly degraded samples (e.g., human DNA recovered from maggot crop contents).

This presentation will impact the forensic science community by providing a new, efficient, and alternate method for analysis of degraded human DNA recovered from maggot crop contents.

Blow flies and flesh flies are well-known for their utility in Postmortem Interval (PMI) estimation, but in some situations they can also provide information on relocation of a corpse, actual source of the maggot, identification of burned remains, and identification of a perpetrator in sexual assault cases.[1-3] This information can be obtained by recovery and analysis of human DNA from maggot crop contents, but recovered human DNA from maggot crop contents tends to be very degraded in a majority of cases. Hence, it generates either incomplete or a total lack of Short Tandem Repeat (STR) profiles.[4] This is primarily because traditional STR kits require longer target DNA fragments for the generation of complete STR profiles. To obtain more information from human nuclear DNA recovered from maggot crop contents, this study utilized InnoTyper™ 21, a novel DNA typing method. InnoTyper™ is a marker system that utilizes a 20 Retrotransposon Insertion Polymorphisms (RIPs) multiplex. Among the advantages of using RIPs are the following: (1) they do not yield stutter artifacts due to slippage during the PCR amplification; (2) there are no known genetic mutations since they are identical by descent only; (3) they are present in very high copy number; and, (4) they have a well-defined genetic lineage that makes them useful for relationship determinations. An innovative primer design allows for the amplicon size for the Alu markers to be reduced to a size (60bp-125bp) that is smaller then currently used STR markers, such that the substantially degraded DNA samples recovered from maggot crops can be analyzed.

To obtain a nuclear profile, DNA was extracted from crop contents of 20 third-instar larvae from two blow fly species (*Calliphora vicina* and *Lucilia sericata*), a positive control (i.e., frozen human liver tissue), a degraded control (30g of the human liver tissue placed under the same conditions as the test samples without exposure to maggots), and a negative control sample using QIAGEN® QIAamp® DNA Investigator kit. Extracted DNA was quantified using *Applied Biosystems® Quantifiler® HP and amplified for 20 RIP loci* and the gender-identifying marker, Amelogenin, using the amplification conditions and run parameters as recommended by the manufacturer. Capillary electrophoresis data were analyzed in GeneMapper® software V4.0.

On average, more than 48% of all alleles (202 out of 420 expected) were successfully recovered by this kit in maggot crops of both fly species. Human DNA recovered from some of the maggot samples actually yielded more complete profiles (90%-100% of all alleles were recovered) than the profiles obtained from the degraded control samples.

In conclusion, InnoTyper™ 21 has huge potential for its utility in the analysis of human DNA recovered from maggot crop contents.

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InnoTyper™, Forensic, Maggot Crop

B146 Differentiation of Sand Grains From Different Locations Using Image Analysis and Multivariate Statistics

Jacob Hock, George Washington University, 2100 Foxhall Road, NW, Washington, DC 20052; and Walter F. Rowe, PhD, George Washington University, Department of Forensic Science, 2100 Foxhall Road, NW, Washington, DC 20007*

After attending this presentation, attendees will understand how to use photomicrography and image analysis coupled with multivariate statistics to compare and differentiate particulate samples from different locales.

This presentation will impact the forensic science community by showing how photomicrography and image analysis along with multivariate statistics can differentiate assemblages of particulate evidence from different sources.

Particulates appear as trace evidence in the investigations of a variety of crimes such as kidnapping, sexual assault, and homicide. Two questions are commonly asked of this type of evidence: (1) where could it have come from; and, (2) could it have come from a specific source? In the case of sand, investigators may want to know the type of environment (desert dune, river, or ocean) from which the sand came. If a crime scene or alibi location has been identified, investigators will inquire whether sand recovered from the clothing of a suspect or the suspect's vehicle could have come from that location. Laboratory examinations of sand samples commonly employ color determinations, X-ray diffraction, and polarized light microscopy to determine mineral content. Sizes, shapes, and textures of sand particles determined by light microscopy and scanning electron microscopy have been found to provide laboratory examiners with useful information about the environment from which the sand grains came.

The goal of this research was to determine if image analysis applied to photomicrographs of assemblages of sand grains could provide numerical estimates of the similarity or dissimilarity of pairs of sand specimens. Sand specimens were obtained from a variety of locations including deserts, rivers, lakes, and ocean beaches. Grab samples of sand grains were permanently mounted on microscope slides and photomicrographs of grains were captured with a 14-megapixel digital eyepiece camera. An open source image analysis program was used to process the images of the sand grains to obtain outlines of individual grains.

After much trial and error, it was determined that the best results were obtained using a mounting medium with a 1.54 refractive index and crossed polarizing filters. Because the sand samples were comprised primarily of quartz, the majority of the sand grains appeared bright against a black background. A montage of images was assembled for each sand sample and processed to obtain the values of a number of attributes of the sand grains, including area, perimeter, Feret diameters, circularity, aspect ratio, roundness, and solidity. Because many of these attributes are inter-correlated, Principal Component Analysis (PCA) was applied to the values of the grain attributes for pairs of sand samples. Factor plots for the first two extracted factors provided visual assessments of the similarity or dissimilarity the two sand samples. The extracted factors from the PCA were used to generate linear discriminant functions for assigning individual sand grains to their correct sample.

Preliminary results suggest that this approach can distinguish sand specimens from different locales. In particular, the desert sand specimen analyzed was very different from the two specimens from rivers, which in turn were different from a sand specimen from an ocean beach. Non-parametric tests (Kolmogorov-Smirnov, Mann-Whitney, and Kruskel-Wallace) were applied to the distributions of the discriminant values for the sand grains in each sample. For all pair-wise comparisons, the null hypothesis that the sand samples were drawn from the same population could be rejected at a confidence level greater than 95%.

These preliminary results demonstrate the potential of a simple, non-destructive method of analysis for the differentiation of particulate forensic evidence from different sources. The size and shape data derived from photomicrography can be combined for PCA with other numerical data, such as visible reflectance spectra and X-ray diffraction patterns for even greater differentiation of samples.

Polarized Light Microscopy, Image Analysis, Principal Component Analysis

B147 Development of Paper Microfluidic Devices for the Detection of Low-Explosives Residue

Kathryn R. Chabaud, BS, Florida International University, 11200 SW 8th Street, Miami, FL 33199; and Bruce R. McCord, PhD, Florida International University, Dept of Chemistry, University Park, Miami, FL 33199*

After attending this presentation, attendees will better understand current research on the development of paper microfluidic devices as a simple and inexpensive alternative to existing presumptive tests for low-explosives residues. Minimal training is required to operate these devices and they are ideal for use in the field by military and law enforcement entities. Attendees will also gain a basic understanding of the metallic components contained in low-explosive devices.

This presentation will impact the forensic science community by providing insight into the possibility of inexpensive, user-friendly, presumptive testing devices for low-explosive residues. This detector could be implemented in pre- and post-blast explosive detection and should be useful in screening unknown materials.

In this project, colorimetric tests are implemented on paper microfluidic devices, permitting metallic residues from low-explosive devices to be detected in the field. Paper microfluidic devices are typically prepared from chromatographic paper creating hydrophilic channels through the use of wax printing followed by lamination at elevated temperatures. Capillary action is then used to mobilize liquids containing dissolved analytes through the wax ink channels of the device. Colorimetric reagents are placed at the terminal end of each channel for detection of the individual analytes, which in this case are metallic salts. Paper-based microfluidic devices were initially designed for application in medicinal and disease testing in remote areas where the lack of refrigeration limits the ability to store expensive reagents. These devices now have a wide variety of applications. Because reagents are dried on the device prior to use, shelf lives are prolonged when compared to liquid reagents. Forensic applications of this technology have been explored. In this study, a paper microfluidic chip has been developed that involves presumptive, colorimetric tests for multiple, different metallic compounds contained in low-explosive residues. Colorimetric tests have been designed for a variety of these components. These tests were first prepared in solution and then optimized for use on paper.

Residue from flash powder-based explosive devices typically consists of inorganic salts resulting from the rapid deflagration of mixtures of inorganic oxidizers and metallic fuels. A paper microfluidic device for the detection of chlorate, perchlorate, and nitrate oxidizers was previously developed. As a follow-up, a paper microfluidic device for the detection of barium, aluminum, iron, and zinc fuels is being developed. Barium is detected via a buffered mixture of sodium rhodizonate, which yields an orange color upon reaction. Aluminum is confirmed via aluminon and ammonium acetate, which yields a pink/red color upon reaction. Iron is detected via p-aminophenol, which yields a purple color upon reaction. Lastly, zinc is detected with dithizone, which yields a bright pink/purple color upon reaction.

These devices are currently undergoing developmental validation to measure the reproducibility, stability, and sensitivity of the analysis. The paper-based devices should prove useful in the analysis of low-explosive residue as the chip is compact and minimal time is needed to produce results. The ultimate goal of the project is to design and test a series of these devices for the presumptive detection of a variety of explosives residues in the field.

Paper Microfluidics, Low Explosives, Colorimetric

B148 Crude Oil Characteristics for Identifying Petroleum Distillates in Fire Debris

Jeanet Hendrikse, MSc, Netherlands Forensic Institute, Laan van Ypenburg 6, The Hague 2497GB, NETHERLANDS*

After attending this presentation, attendees will understand which crude oil characteristics are necessary to distinguish petroleum distillates from non-conventional "petroleum-like" products in fire debris analysis results.

This presentation will impact the forensic science community by providing more detailed guidance in identifying and differentiating certain ignitable liquids products than can currently be found in scientific literature.

Petroleum distillates are crude oil refining fractions from the first refining process, the atmospheric distillation. These so called "straight-run" or "conventional" distillates are characterized by dominating alkanes and a relatively low content of aromatics. The composition of distillates generally mirrors the composition of the crude oil(s) from which it was distilled. The crude oil characteristics can therefore be used by fire debris experts for identifying petroleum distillates in fire debris and to distinguish them from: (1) distillates that have been further refined; and, (2) "petroleum-like" products that have been produced synthetically.

Straight-run distillates can be further refined for specific applications. Examples of processes are de-aromatization and de-waxing. De-aromatization is employed to remove or reduce the aromatic content; the alkane composition in this process remains unaffected. De-waxing is used to meet the low temperature properties required for its application. The end product of this process has no (or a reduced level of) n-alkanes and a branched alkane fingerprint pattern that no longer resembles that of crude oil. An alternative classification for these products, when encountered in fire debris analysis results, must therefore be considered.

Due to the imminent depletion of oil and the effect its usage has on the environment, more and more "petroleum-like" products are currently produced synthetically. Examples of such products are Gas-To-Liquid (GTL) diesel, some lamp oils, and some lighter fluids. GTL diesel is (and the other examples can be) the end product of the so called Fisher-Tropsch (FT) process. Some products, like lamp oils, are also currently known to be the blend result of a normal alkane product and an isoparaffinic product, the latter of which is obtained, for example, through an alkylation process.

Compared to petroleum distillates, these synthetic "petroleum-like" products do not contain a characteristic crude oil fingerprint pattern: branched alkanes are present but their pattern is different from that of crude oil, and both cycloalkanes, isoprenoïds, and aromatics (all present in crude oil) are absent. These synthetic "petroleum-like" products fall into the American Society for Testing and Materials (ASTM) E1618 class "Others-Miscellaneous."

The alkane pattern that is considered characteristic for straight-run petroleum distillates is discussed and illustrated in this presentation. Crude oil examples are illustrated to show that these characteristics originate from crude oil. Examples of more refined distillates and of synthetic "petroleum-like" products are illustrated to show that the alkane fingerprint pattern of these products does not resemble/no longer resembles that of crude oil.

Crude Oil, Characteristic, Petroleum Distillate

B149 Forensic Analysis of Textile Fibers Exposed to Laundry Detergents Using Fluorescence Excitation-Emission Spectroscopy

Nirvani Mujumdar, MS, 12029 Solon Drive, Apt 105, Orlando, FL 32826*

After attending this presentation, attendees will better understand forensic spectroscopy as a non-destructive analytical technique for forensic trace analysis.

This presentation will impact the forensic science community by demonstrating the usefulness of forensic spectroscopy as a non-destructive analytical technique for forensic trace analysis. This presentation will help attendees distinguish between fibers exposed to multiple launderings versus those that were never exposed. This study seeks to explain the effects of repetitive laundering on the total fluorescence of fibers and to determine the number of washes essential to make distinctions between washed and unwashed fibers.

Textile fibers are a major form of trace evidence, and the ability to reliably classify them is useful for forensic scientists. Before becoming an important source of evidence, fibers discovered at a crime scene are likely to be exposed to multiple launderings, which can possibly further add characteristic fluorescence components on the fibers. This can be a useful tool for the identification and analysis of forensic fiber evidence since detergents usually contain fluorescence whitening agents, which when accumulated on the fibers can modify the original spectral features of these fibers.

The primary goal of this project was to be able to distinguish between fibers exposed to multiple launderings versus those that were never exposed. Discovering common origins of trace evidential textile fibers can be a challenging task when fiber structure or dye composition does not provide exclusive identifying information. Introduction of new chemical species following mass production and distribution of a textile may be exploited to trace its history and identify the origin of its fibers.

In this presentation, a non-destructive technique known as fluorescence excitation-emission spectroscopy was used to examine the alteration in the fluorescence spectral fingerprint of single fibers resulting from exposure to commonly used detergents that contain fluorescent whitening agents. Seven top-selling detergents were used to wash fibers composed of diverse classes of dyes, colors, and compositions. Undyed acrylic, cotton, and nylon fabrics dyed respectively with basic green 4, direct blue 1, and acid yellow 17 dyes were laundered up to six times with the selected detergents, and the spectral contribution of each detergent onto single fibers was quantified and shown to reach a maximum after five sequential washes; however, in certain cases, some detergents even showed statistically meaningful differences to the fiber spectra after only a single wash. Fluorescence emission spectral profiles were collected and principle component cluster analysis was employed for additional statistical comparisons and differentiations, which facilitated to determine that the spectra of washed fibers were distinct from those of dyed, unwashed fibers. In the case of dyed nylon and cotton fibers, the laundered fibers were never misclassified as unwashed, but acrylic fibers showed very little detergent contribution to the fluorescence spectra.

Through this study, an attempt has been made to explain the effects of repetitive laundering on the total fluorescence of fibers and also to determine the number of washes essential to make distinctions between washed and unwashed fibers. Collection of a library of spectra from fibers washed zero to six times with popularly utilized detergents has enabled characterization of the spectral contribution of the detergent to the overall spectrum of the fiber, dye, and detergent. Lastly, these results indicate that washed and unwashed fibers were distinguishable with 95% accuracy.

Forensic Fiber Analysis, Fluorescence Spectroscopy, Laundry Detergents

B150 Investigative Predictions of Smokeless Powder Manufacturers

Dana-Marie K. Dennis, PhD, 5241 Millenia Boulevard, Apt 203, Orlando, FL 32839; Mary R. Williams, MS, National Center for Forensic Science, PO Box 162367, Orlando, FL 32816-2367; and Michael E. Sigman, PhD, University of Central Florida, National Center for Forensic Science, PO Box 162367, Orlando, FL 32816*

After attending this presentation, attendees will understand how the application of a Bayesian network to smokeless powder's physical and chemical characteristics data from a database can provide probability-based estimates of the manufacturer.

This presentation will impact the forensic science community by providing an investigative tool with the ability to predict the manufacturer of a smokeless powder based on its physical and chemical properties.

This research proposes the use of a Bayesian network that utilizes data from the National Center for Forensic Science (NCFS) and the Technical/Scientific Working Group for Fire and Explosions (T/SWGFEX) Smokeless Powders Database to suggest, by means of posterior probabilities, the manufacturer of a smokeless powder based on its physical and chemical characteristics. The dataset, comprising 119 NCFS smokeless powder database samples, contains information pertaining to the manufacturer, color, shape, average diameter, average length, and six class-associated ions which were previously determined from cluster analysis. Bayesian network structures are referred to as Directed Acyclic Graphs (DAG), which are comprised of nodes and arcs. Nodes represent events or variables which can have multiple states. Arcs represent causal dependencies between the nodes and are directed from parent nodes to child nodes. Probability tables are associated with each node; a parent node table encodes prior probabilities and child node tables encode class-conditional probabilities.

Two network designs have been explored. The first network contains a parent node representing the manufacturer of the smokeless powder with nine child nodes representing color, shape, m/z46, m/z120, m/z134, m/z149, m/z165, and m/z169. The second network is similar to the first, but has two additional child nodes representing the average diameter and average length of the smokeless powder. The m/z ions characterize the chemical composition of the smokeless powder; each one is a base ion for a specific compound or group of compounds. The networks were constructed and manufacturer posterior probabilities calculated using R statistical computing software. Prediction performance was estimated based on 100 iterations in which each repetition randomly withheld 5% of the dataset for testing; the remaining 95% was used to train the network. Values in the probability tables were calculated based on frequencies in the training dataset.

The networks were instantiated by entering evidence in the child nodes for each smokeless powder sample in the test set, and the prior probabilities in the manufacturer node were updated to posterior probabilities after the evidence was observed. The manufacturer for each test sample was predicted based on the highest posterior probability. Overall percent correct rates were determined by calculating the number of correct predictions; that is, where the known and predicted manufacturer were the same. The networks achieved overall percent correct rates of 66% and 70% for NET 1 and NET 2, respectively. The overall percent correct rates are significantly larger than the 20% random assignment rate. Work is continuing to focus on improving the overall percent correct based on posterior probabilities.

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Smokeless Powders, Bayesian Networks, Database

B151 Modern Methodology for Explosives Tagging and Encoding Based on Luminescent Metal Organic Frameworks

Filipe Gabriel B. Mauricio, MSc, University of Brasilia, Instituto de Química - Campus Darcy Ribeiro, Brasília 70910-000, BRAZIL; Ingrid T. Weber, PhD, University of Brasilia, Instituto de Química -Campus Darcy Ribeiro, Caixa Postal 04478, Brasilia, Distrito Federal (DF) 70910-000, BRAZIL; Adauto Z. Pralon, MSc, Federal Police Departament, SAS Quadra 6, lotes 09/10 - ED.SEDE/DPF, Brasília 70037.900, BRAZIL; and Marcio Talhavini, PhD, Federal Police Departament, SAS Quadra 6, lotes 09/10 - ED.SEDE/DPF, Brasília 70910-000, BRAZIL*

After attending this presentation, attendees will understand methods of post-blast identification, collection of explosive residues by the addition of luminescent taggants, and the possibilities of encoding based on the metal ratio of the lanthanide ions by Energy Dispersive Spectroscopy (EDS) and typical light emission.

This presentation will impact the forensic science community by providing an easy, fast, and unequivocal method of identification/collection of explosive residues by the addition of luminescent taggants in commercial explosives that act not only as an optical probe, but that provide an encoding based on the metal ratio. The information provided by this presentation can be used to offer evidence that allows the identification of suspects in the criminal use of explosives.

During the past several years, Brazilian authorities have reported an increase in the number of attacks throughout the country on Automated Teller Machines (ATMs) with the use of explosives. According to the eighth edition of the national survey on bank attacks in Brazil, an increase of 147% was observed between the years 2011 and 2014.[1] Concomitantly, a growth of 38% in the cases of deaths by terrorist attacks has been observed around the world, in accordance with the Global Terrorism Index, in which 49% of these correspond to explosives attacks.[2] The evidence indicates the use of Ammonium Nitrate-Fuel Oil (ANFO) obtained from miners and carries in the attacks on ATMs in Brazil.[3]

Tracking and identifying suspects after explosions is one of the most challenging tasks in the forensic field. After detonation, most of the residue of the explosives is lost due to the volatility of the material, with the remaining trace residues being spread over a large debris field.[4] The growing criminal use of explosives has driven authorities to seek technologies that allow tracking of explosives. In 1973, Ryan et al. proposed to include a miscellany of metals and oxides as a way to encode explosives; luminescent materials were incorporated in these taggants in order to make the visualization and collection of residues easier for analysis.[5] Although good results have been obtained for the identification of residues, the need for including additives in explosive and encapsulating taggants was reported, which rendered the process less attractive.[6-9]

This work proposes a method to tag explosives by the direct addition of a luminescent Metal Organic Framework (MOF), which acts simultaneously as an optical probe (allowing easier visualization and collection of explosive residues) and as a chemical taggant (which tracks the explosive's origin). This research reports the use of two isostructural MOFs, (La0.8Tb0.2)(DPA)3(H2O)3 and (La1.6Eu0.2Tb0.2)(DPA)3(H2O)3, synthetized hydrothermally that emit orange and green light, respectively. The taggants were incorporated directly and manually into samples of ANFO explosives. Charges of 10g of tagged explosives were attached and detonated in computer cabinets simulating conventional ATMs.

Post-explosion residues were visually observed *in situ*, using only an Ultraviolet (UV) lamp (254nm). Afterward, the residues were collected and identified by luminescence spectroscopy and Energy Dispersive Spectroscopy (EDS). The EDS metal ratio of the explosives showed a high precision when compared with the pure samples that allowed an unequivocal coding method for each taggant. It was possible to not only observe the luminescent residues at the explosion scene, but the emitted color coupled to metal ratio also allowed for the unequivocal identification of the taggants. The methodology proved to be easy, fast, and very reliable.

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Post-Blast, Taggant, Explosive

B152 Do the Bulk Area and the Exterior Surface of Modern Container Glass Exhibit Differences in Refractive Index (RI) Measurements?

Joseph Insana, West Virginia University, Dept of Forensic and Investigative Science, 304 Oglebay Hall, PO Box 6121, Morgantown, WV 26506-6121; and Patrick Buzzini, PhD, Sam Houston State University, Chemistry/Forensic Science Bldg, 1003 Bowers Boulevard, Box 2525, Huntsville, TX 77314*

After attending this presentation, attendees will better understand the potential differences of RI measurements observed between external surfaces and bulk areas of glass containers. Attendees will also learn about the implementation of a simple and fast method to sample glass fragments from the surface of a glass object.

This presentation will impact the forensic science community, with emphasis to trace evidence examiners, by providing updated information on the potential RI differences that characterize the heterogeneity of this important optical property within modern container glass.

RI is known to be a highly discriminatory property used in forensic comparative examinations of glass. A critical aspect of RI measurements is the evaluation of intra-source variation. Indeed, RI is known to vary at different locations of a given glass object. In addition to spatial heterogeneity, previous studies indicate that differences in RI measurements could be observed between the external surface and the bulk area of a glass object. Considering the improvements of modern glass manufacturing processes, this study compares RI data from the external surfaces of glass containers to those collected from their bulk in order to determine if a significant difference exists.

The objectives of this study were: (1) the development of a method that separates the exterior surface from the internal bulk; (2) to produce representative RI data from the selected glass containers; and, (3) to apply a simple and robust statistic that informs about a potential difference between RI data from the bulk and exterior of a given glass container. This study intends to provide objective information to glass examiners concerned with the understanding of RI variation that could be expected between bulk and external surface for container glass. These results can be valuable when examiners interpret potential differences observed during comparative examinations or when they attempt to explain the dispersion of RI data as a consequence of a sampling method.

The body areas of eight glass containers were selected as initial samples for this study. These were two similar green beer bottles, two similar brown beer bottles, two similar green wine bottles, and two similar colorless honey pots. A novel methodology was developed to isolate the surface layer of glass fragments from their bulk. It consists of a simple and fast approach that scrapes fine glass debris from the surface of a glass fragment. Glass fragments were collected from the body area of the selected containers (as opposed to neck and base). A total of 560 measurements were taken using the traditional method for Glass Refractive Index Measurement based on the joint use of a hot stage and phase contrast microscopy. Fourteen fragments were selected for each container, seven from bulk and seven from exterior. Five RI measurements per fragment were taken for a total of 70 RI measurements per container. Data from the two areas were compared using the Welch version of the Student *t*-test (given the observed differences of variance between the two compared distributions). Differences between bulk and exterior were observed from the body areas of three glass containers out of eight. These differences were observed from glass objects of the same type and manufacturer.

Trace Evidence, Glass, Refractive Index

B153 Comparison of Cosmetic Foundations by Analysis of Preservative Content Using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Thomas A. Brettell, PhD, Cedar Crest College, 100 College Drive, Allentown, PA 18104; and Emily A. Myers, BS, 2133 Stoops Court, PO Box 602, North Apollo, PA 15673*

After attending this presentation, attendees will have a better understanding of how the analysis of preservatives can be used to compare cosmetic foundation samples submitted as trace evidence exemplars to forensic science laboratories.

This presentation will impact the forensic science community by providing a simple and sensitive LC/MS/MS method to compare cosmetic foundations by the analysis of a specific set of preservatives found in most cosmetic samples.

Preservatives are natural or synthetic ingredients that are commonly added to products in order to prevent spoilage, including but not limited to microbial growth or undesirable chemical changes, ultimately extending the product's shelf life. Without the addition of preservatives, the foundation has the ability to easily become contaminated, leading to product degradation and increasing the risk of irritation or infection. In the United States, the Food and Drug Administration regulates the use of preservatives under the cosmetic provisions of the law and requires manufacturers to determine at what levels the preservatives are considered "safe" for consumer use. The most widely used preservatives in cosmetic products are parabens.

This study developed an LC/MS/MS method for differentiating different brands of cosmetic foundations by observing the absence and or presence of specific preservatives, including six different parabens. The analyte preservatives used in this study were methylparaben, ethylparaben, n-propylparaben, isopropylparaben, butylparaben, benzylparaben, tocopheryl acetate, and 3,5-di-tert-butyl-4-hydroxytoluene (BHT). The method is capable of separating and identifying all eight preservatives in less than seven minutes including the separation of n-propylparaben and isopropylparaben, which has not been accomplished and reported prior to this method. LC/MS/MS data was acquired using an ABI® SCIEX™ 3200 QTRAP® triple quadrupole mass spectrometer interfaced with a Shimadzu® LC system. The instrument utilized Electrospray Ionization (ESI) and all samples were run in positive-ion mode monitoring. Chromatography was performed on a 5.0cm x 3.0mm x 2.7µm Ultra® biphenyl column. The strong mobile phase used was 0.1% formic acid in 2-propanol and the weak mobile phase used was 0.1% formic acid in High-Performance Liquid Chromatography (HPLC) -grade methanol. A Shimadzu® SIL-20AC Prominence auto sampler injected 2.0µL of sample and the column oven temperature was set isothermally at 25°C throughout the run with a flow rate of 0.300µL/min. A retention time optimization study provided preeminent separation conditions.

Foundation samples were prepared by adding 100mg of each foundation to 5mL of methanol:acetonitrile (1:1) and sonicating for ten minutes. After sonication, the solution was placed into centrifuge tubes and centrifuged for five minutes at 800G. After centrifugation, the supernatant was carefully removed using disposable pipettes and filtered using 0.2µm Millipore® filters. Then 1mL of the supernatant was added to a vial along with 60µL of the internal standard (BHA). Lastly, 2.0µL of sample was injected onto the LC column.

Separating and identifying the six parabens as well as the other preservatives proved to be simple and quick. The method is capable of identifying which preservatives are present in a cosmetic sample with a limit of detection of 0.5µg/mL. Twenty-six different brands of cosmetic foundations were tested and easily differentiated by analysis of the preservatives in the samples using the LC/MS/MS method.

Preservatives, Cosmetics, LC/MS/MS

B154 Development of a Sample Clean-Up Procedure for the Recovery of Trace Quantities of Organic Explosives in Soil and Sand

Erin Waddell, PhD, 2501 Investigation Parkway, Quantico, VA 22135; Jennifer Thomas, PhD, 3110 Spring Drive, Alexandria, VA 22306; Christopher C. Donnelly, 2121 Aquia Drive, Stafford, VA 22554; and Mark L. Miller, PhD, FBI Lab, CFSRU, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, attendees will be informed about techniques for the extraction and detection of trace amounts of organic explosives residue from soil, sand, and oil-contaminated soil matrices utilizing Solid Phase Extraction (SPE).

This presentation will impact the forensic science community by providing an improved clean-up method for detecting trace organic explosives residue in complex matrices. In addition, this presentation will compare and contrast three SPE cartridges (Bond Elut NEXUS®, Empore™ SDB-XC, and Oasis® HLB) based on the recovery of organic explosives residue from soil/sand and matrix rejection.

The forensic science community is constantly searching to improve currently established protocols in lieu of older alternatives. A fast and simple option often used for samples is to use syringe filtration of the extract prior to sample analysis. Although samples are filtered, the sample typically contains matrix interferences that are not removed and are injected with the sample into the instrument. In this research, a Gas Chromatograph coupled to an Electron Capture Detector (GC/ECD) was utilized for analysis. The matrix of filtered samples may lead to signal suppression, increased background noise, and extra (or missing) peaks. If this occurs, instrument maintenance must be performed, which can be costly both in time and materials. The research to be presented focuses on finding an efficient extraction process that can separate the analyte(s) from the contaminants, facilitating sample throughput and reducing maintenance costs associated with instrument malfunction due to damage from matrix interferences.

Twelve organic explosives were investigated in this study: ethyleneglycol dinitrate (EGDN); dimethyl dinitrobutane (DMDNB); 4-nitrotoluene (4-NT); nitroglycerin (NG); 2,4-dinitrotoluene (DNT); 2,4,6-trinitrotoluene (TNT); pentaerythritol tetranitrate (PETN); trimethylene trinitramine (RDX); 2,4,6-trinitrophenylmethylnitramine (Tetryl); tetramethylene tetranitramine (HMX); erythritol tetranitrate (ETN); and cyclotrimethylene trinitrosoamine (R-salt). A mixture of these explosives was spiked onto samples of soil, sand, and soil contaminated with used motor oil. Acetonitrile was used to extract the explosives from the matrices. As a comparison of a simplified extraction procedure, syringe filtration was used prior to GC/ECD analysis for one sample set. This involved filtering the extracted liquid, a dry-down step to reduce sample volume, and injection via syringe for analysis by GC/ECD. In this research, the extraction liquid was cleaned up using Solid Phase Extraction (SPE) prior to GC/ECD analysis. This method has the advantage of bypassing the time-consuming dry-down step and of removing residual impurities contributed by the complex matrix.

In conclusion, the experiments reflected that it was possible to extract organic explosives residue from soil and sand samples using acetonitrile. Using SPE, it was possible to clean up the samples for GC/ECD analysis. Preliminary results indicate that the SPE cartridges provided the recovery of all 12 explosives, purified the oil-contaminated samples well, and generally did not require an extensive processing time. The results also indicate that the Oasis® HLB cartridges provided the highest percent recoveries of the explosives and are the most cost effective.

Explosives, Trace Analysis, Solid Phase Extraction

B155 Analysis of Inks Via a Microfluidics Extraction Device With a Quadrupole Time-of-Flight Mass Spectrometer (qTOF/MS)

Emily Lichtenberger, BS, North Carolina State University, 2401 Research Drive, Raleigh, NC 27606; and Nelson R. Vinueza, PhD, North Carolina Sate University, 2401 Research Drive, Campus Box 8301, Raleigh, NC 27695*

After attending this presentation, attendees will better understand a new Microfluidics Device (MFD) that can facilitate extraction of dyes, inks, etc. as well as a new understanding of analyzing inks from questioned documents via tandem mass spectrometry. Originally, the MFD was used for the extraction of dyes from fibers with minimal room for error because of the lack of contact between the examiner and the evidence.[1] Proven as a useful tool in previous work, the use of the MFD has expanded to analgesic tablet analysis and now to ink analysis from questioned documents, the main focus of this presentation.

This presentation will impact the forensic science community by providing a fast, accurate, and reproducible extraction and analysis methodology for a variety of evidences with minimal areas for human error and minimal damage to the evidence. For MFD Mass Spectrometric (MS) analysis, only a small amount of the evidence is needed, whether it is a single fiber a couple millimeters in length from a piece of fabric or a small microchip of the questioned document. This ensures that the majority of the evidence is preserved for court proceedings.

In these experiments, a variety of pens, markers, and highlighters in colors such as red, orange, yellow, and pink were analyzed using MFD-MS via Agilent® Technologies' 6520 quadrupole Time-of-Flight (qTOF). From previous work, it is known that Rhodamine 6G (Rh6G) and Rhodamine B (RhB) are common dyes found in writing utensils of these colors.[2,3] Both of these compounds were purchased from Sigma Aldrich® and used as standards for reference. The six writing utensils analyzed were obtained from a variety of manufacturers such as Sharpie® and Office Depot®. All experiments were completed at North Carolina State University in the analytical chemistry laboratory of the College of Textiles in Raleigh, NC.

Sample preparation consisted of drawing a single line to represent writing on a questioned document. A Harris Micro-Punch tool with a 2.0mm hole was used to take a micro-punch of the document. Due to markers having a larger amount of dispensed ink than pens such as ball point pens, only about a third of the micro-punch taken contained ink to ensure that saturation of the mass spectrometer detector would not occur, which indicates that more of the writing is preserved on the document. The micro-punch was then placed in the sample chip and inserted into the MFD. The extraction proceeded within the MFD using the program created in-house.[1] Acetonitrile (ACN) was flushed into the chamber of the cavity containing the micro-punch to extract the ink from the paper. This was done four times to ensure that extraction had occurred. Mass spectra were obtained via the qTOF mass spectrometer. The exact mass was identical for both Rh6G and RhB (443.2329); however, their structures differed and could be differentiated by their fragments when targeted via Tandem MS (MS/MS). RhB fragmentation included the loss of -CO2 which was observed by a fragment ion with m/z of 399; Rh6G fragmentation included the loss of -C2H4 which was observed by a fragment ion of m/z of 415.[3] This identification can be used to determine if one or both rhodamines are in the extracted ink. After each sample was analyzed, the MFD/MS system was flushed with Isopropyl Alcohol (IPA) until the ions of interest were low in abundance, and a blank sample was recorded to ensure that signals observed with the samples were from the extracted dyes. Total analysis and cleaning time per sample was estimated to be approximately 15-20 minutes depending on the user's knowledge of the system.

In conclusion, this analysis system in conjunction with the use of Rh6G and RhB as standards for red, orange, yellow, and pink writing utensils provides a new methodology to analyze questioned documents. Determination of whether the markers contained the rhodamines and, if so, which ones, have been successful and through repetition have shown that this technique is accurate and reproducible. Analysis time is decreased exponentially and minimal damage is done to the questioned document, ensuring that the majority of the evidence is conserved for later use.

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Ink Analysis, Mass Spectrometry, Microfluidics

B156 Gas Chromatography/Mass Spectrometry (GC/MS) Measurement of Gasoline Vapor Absorption on Clothing in a Confined Space

Charles R. Cornett, PhD, University of Wisconsin-Platteville, Dept of Chemistry, 1 University Plaza, Platteville, WI 53818; Sara C. Karp, BS, University of Wisconsin-Platteville, 845 S Chestnut Street, Apt 107W, Platteville, WI 53818; Ruth M. Henk, BS, Wisconsin State Crime Laboratory-Milwaukee, 1578 S 11th, Milwaukee, WI 53204; Kristy Stowe, BS, University of Wisconsin-Platteville, Dept of Chemistry, 1 University Plaza, Platteville, WI 53818; and Raymond G. Lenz, BS, Wisconsin State Crime Laboratory-Milwaukee, 1578 S 11th, Milwaukee, WI 53204*

After attending this presentation, attendees will better understand gasoline vapor absorption on cotton clothing in a confined space.

This presentation will impact the forensic science community by adding to the body of knowledge regarding the transfer of gasoline vapors to clothing in a confined space, information particularly important to the ignitable liquid/arson debris analysis community.

A recent case produced a question regarding the possible deposition of gasoline vapors on clothing as a suspect walked across a room in which the vapors were present. In the course of testimony, the defense council noted that gasoline was present on the defendant's footwear, yet there was no determination of gasoline on the defendant's clothing.

This research project focuses on the absorption of gasoline vapors by clothing in confined spaces. Research by Folkman et al., Coulson et al., and Errata et al. (under review) primarily deals with splashing or spilling of ignitable liquids onto clothing and the persistence of sufficient concentrations of ignitable liquids for identification.[1-3]

The experimental process was as follows. New, never-laundered, same-sourced cotton T-shirts were purchased along with new, one-gallon paint cans. An unventilated, steel, flammable 1.6m x 0.8m x 0.5m cabinet containing approximately 30L of 87-octane gasoline in standard one-gallon gasoline containers was used as the source of gasoline fumes. Vapors were allowed to accumulate in the cabinet for three months prior to the experiment. Samples were taken in triplicate of cotton T-shirts stored inside the cabinet for six hours, one hour, and fifteen minutes. In addition, one experimental condition measured the amount of absorbed gasoline vapor after 15 minutes of exposure followed by a one-hour ventilation in a fume hood. Subsequently, an additional experiment was performed on three T-shirts left hanging one meter from the open doors of the cabinet. Each T-shirt was placed in a clean, new, one-gallon paint can. An activated charcoal strip was suspended in the can, which was then heated at 70°C for 16 hours. The activated charcoal strips were washed with carbon disulfide and analyzed by GC/MS.

Results of the experiment show detectable amounts of gasoline (BET, alkylbenzenes, and naphthalenes) for the T-shirts exposed to gasoline vapors in the cabinet for periods of six hours and one hour; however, even in the extremely confined and unventilated space, the T-shirts exposed for 15 minutes did not display sufficient naphthalene concentrations for an identification, even prior to the one-hour, in-hood ventilation. The conclusion is that the necessary concentration for gasoline vapors to absorb into cotton T-shirts and be detected would likely lead to the incapacitation or death of the individual wearing the clothing.[4] The absorption and persistence of solely gasoline vapors onto cotton clothing of a suspect who has left the crime scene appears very unlikely according to the results of these experiments.

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Gasoline, Ignitable Liquid, Clothing

B157 Observations on the Incidence of Transfer of Fibers to Knives During Penetration Cuts

Barbara Doupe, MSc, Centre of Forensic Sciences, 25 Morton Shulman Avenue, Toronto, ON M3M 0B1, CANADA; Vanessa Londero, BSc, University of Ontario Institute of Technology, 2000 Simcoe Street, N, Oshawa, ON L1H 7K4, CANADA; and Cecilia Hageman, PhD, University of Ontario Institute of Technology, 2000 Simcoe Street, N, Faculty of Science, Oshawa, ON L1H 7K4, CANADA*

After attending this presentation, attendees will have a better understanding of fiber transfer to knives during stabbings through clothing. Possible correlations were examined between the number of fibers transferred and their location on a knife blade versus the blade type, number of penetrations, direction of penetration, fabric construction, and fiber composition.

This presentation will impact the forensic science community by dispelling misconceptions about fiber transfer onto knife blades during stabbing incidents. This includes the expectation that serrated blades are significantly more prone to entrain and retain fibers than straight blades and that the fiber composition and/or fabric construction would significantly affect the number of fibers transferred. Fibers transferred to a knife blade can be critical evidence when the knife is recovered in a different location from the complainant and DNA is not probative to the case. While previous studies address the force required for a knife to penetrate a model of a clothed body or the fabric damage caused, there have not been previous studies looking at either the transfer of fibers to a knife during stabbing nor the variables that may impact the number and location of transferred fibers.

Pork shoulders were used as the knife penetration substrate. They were covered with four different types of fabric to mimic clothing: cotton-woven denim fabric, cotton jersey knit fabric, polyester tricot knit fabric, and polyester-woven windbreaker fabric. Single and double penetrations were made aligning the knife blade with the lengthwise direction, the crosswise direction, or on a diagonal of the fabric. The penetration cuts were made using either a serrated blade or a straight blade. Each sequence of variables was repeated three times for a total of 144 penetrations. After each penetration, the fibers transferred to the blade were collected and counted.

The average number of fibers collected after single penetrations with the serrated blade and with the straight blade were, respectively, 98 and 86 for the cotton denim fabric, 92 and 32 for the cotton jersey knit fabric, 80 and 105 for the polyester tricot knit fabric, and 54 and 29 for the polyester windbreaker fabric. These fibers were most frequently observed along the cutting edge and along the deepest point of penetration on both types of blades.

The average number of fibers collected after double penetration with the serrated blade and with the straight blade were, respectively, 102 and 80 for the cotton denim fabric, 72 and 63 for the cotton jersey knit fabric, 37 and 76 for the polyester tricot knit fabric, and 69 and 38 for the polyester windbreaker fabric. These fibers were most frequently observed along the deepest point of penetration after the double penetrations on the straight blade; however, this was not always the case for the serrated blade.

Significant numbers of fibers were transferred to the blades along the cutting edge and at the deepest point of penetration of the blade during penetration. Correlations were not observed between the number of fibers transferred, or their location on the blade, with blade type, number of penetrations, direction of penetration, fabric construction, or fiber composition. The expectation that more fibers would be transferred to a serrated knife was not supported by the observations.

Fiber, Transfer, Knife Cuts

B158 Low-Cost Lanthanide-Organic Framework Markers for Gunshot Residue (GSR) Identification

Isabela Bastos Serwy, SQN 305 Bl C Ap 305 Asa Norte Brasília DF, Brasília, Distrito Federal 70737060, BRAZIL; Kaline Wanderley, PhD, Chemistry Institute, University of Brasília, 709, Brasília, BRAZIL; Marcella Auxiliadora de Melo Lucena, MS*, Rua Capitão José Nogueira Costa, 46, Várzea, Recife, Pernambuco 50810-270, BRAZIL; Marcio Talhavini, PhD, Federal Police Departament, SAS Quadra 6, lotes 09/10 - ED.SEDE/DPF, Brasília 70910-000, BRAZIL; Marcelo O. Roderigues, PhD, LIMA, Chemistry Institute, University of Brasília, Brasília, BRAZIL; and Ingrid T. Weber, PhD*, University of Brasilia, Instituto de Química - Campus Darcy Ribeiro, Caixa Postal 04478, Brasilia, Distrito Federal (DF) 70910-000, BRAZIL*

After attending this presentation, attendees will better understand the new types of low-cost markers for non-toxic ammunition using luminescent materials, which simplify the investigative routines but also allow the use of the characterization method already used for conventional munitions.

This presentation will impact the forensic science community by adding to research by providing the synthesis of a new type of luminescent markers for GSR based on the Metal-Organic Framework (MOF) [Zn(BDC)(H2O)2]n doped with the lanthanide ions terbium and europium, by a simple, fast, and reliable methodology with low-cost production.

GSRs are an important source of information in forensic analysis; however, with the advent of Non-Toxic Ammunition (NTA), also known as lead-free ammunition, the characterization of GSR became a very difficult task since GSR produced by NTA does not contain any characteristic elements (for example, Pb, Sb, and Ba) that allow unequivocal characterization. Thus, the standard methodology for GSR characterization adopted by the American Society for Testing and Materials (ASTM) International based on Scanning Electron Microscopy coupled to Energy Dispersive Spectroscopy (SEM/EDS) becomes inadequate. In this context, luminescent ammunition markers were developed. These markers allowed the visual identification of the Luminescent GSR (LGSR) on the shooters' hands, the firearm, and at a simulated crime scene using only an Ultraviolet (UV) lamp. It provides a simple, fast, and reliable methodology that improves the crime scene investigation.[1-3]

Among many materials tested as possible markers, the MOFs containing lanthanide ions were shown to have a large potential to be used as GSR markers due to their high luminescence and high chemical and thermal stability. Excellent results were obtained with lanthanide-based MOFs; however, in general lanthanide-based materials are relatively expensive.[2,3] On the other hand, the cost of the markers can be reduced by using d-metal-based MOFs doped with lanthanides. Thus, in this work, the coordination network [Zn(BDC)(H2O)2]n doped with different proportions (0.01% to 100%) of Tb^{3+} or Eu^{3+} evaluated as GSR luminescent markers. The materials were synthetized at room temperature by direct precipitation reaction and incorporated into the gunpowder of 9mm CleanRange® NTA cartridges in a ratio weight of 4%. Then, for each marker, three shots were performed using Glock® G17 pistols at the indoor shooting range in the ballistics service of the National Institute of Criminalistics of the Brazilian Federal Police (NIC/BFP). Next, LGSR particles were visualized on the firearm, the shooter's hands, and the target when MOFs containing more than 1% of lanthanide were used. The best results were obtained when 10% of Tb^{3+} or Eu^{3+} were incorporated into the MOF. Finally, the LGSR were collected with stubs covered with double-sided adhesive conductive carbon tape and analyzed by SEM/EDS and by video spectral comparator (where emission spectra were acquired).

The emission spectra of LGSR showed the characteristic transitions $^5D_4 \rightarrow {}^7F_J$ (J=3-6) of Tb^{+3} and $^5D_0 \rightarrow {}^7F_J$ (J=0-4) of Eu^{+3}, with the most intense the transitions at 543nm and 614nm (responsible for the green and red emission colors of these markers, respectively). Additionally, the EDS spectra confirmed the concomitant presence of the zinc and Tb or Eu ions in the LGSR, showing that in addition to the optical signature, the markers confer a chemical signature to ammunition. $[Zn(BDC)(H_2O)_2]n$: Tb or Eu presents an interesting effective cost, being up to eight times less expensive than the fully lanthanide-based marker.

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Luminescent Marker, Metal-Organic Framework (MOF), Gunshot Residue

B159 Characterization of Hair Dyes Using Ultra High-Performance Liquid Chromatography Electrospray Ionization Time-of-Flight Mass Spectrometry (UHPLC-ESI-TOF/MS) for the Forensic Analysis of Dyed Hair

Diana I. Camacho, 3819 S Honore, Chicago, IL 60609; Ira S. Lurie, PhD, George Washington University, Dept of Forensic Science, 2100 Foxhall Road, NW, Somers Hall, Lower Level, Washington, DC 20007; and Ioan Marginean, PhD, George Washington University, 2100 Foxhall Road, NW, Somers Hall L14C, Washington, DC 20007*

After attending this presentation, attendees will learn about the process and chemicals used to dye human hair and options for analysis of dyed hair. The attendees will be exposed to the idea of using HPLC with MS detection to analyze dyed hair for forensic purposes.

The presentation will impact the forensic science community by suggesting a technique that brings additional support to hair evidence when an association can be made between a person and the crime scene based on dyed hair. This technique involves dye extraction from hair using typical solvents followed by LC analysis with MS detection.

Forensic analysis of questioned hair typically entails microscopic comparison with known hair collected from the suspect or from the victim possibly followed by mitochondrial DNA analysis if the investigation microscopic comparison does not result in an exclusion. The microscopic comparison is a quick yet notoriously unreliable technique, which requires an extremely well-trained investigator. Many convictions obtained using hair evidence were later reversed based on DNA evidence, emphasizing an unacceptable rate of false positives.

Mitochondrial DNA analysis can reduce the probability of false positives to a very low, yet still unacceptable, value. Unlike nuclear DNA analysis, mitochondrial DNA analysis cannot individualize the evidence to a single suspect/victim with sufficient confidence. When both the questioned and the known hairs were dyed, the analysis of hair color can further increase the evidentiary power of hair. This is especially important when any other type of evidence linking the victim/suspect to the crime scene is missing.

Eighty percent of commercial hair formulations are permanent, oxidative dyes. They consist of a mixture of small precursors known as primary intermediates and coupling molecules that enter the hair fiber and react under oxidative conditions to form larger dye molecules. The size of the dyes prevents them from leaving the hair and they become deposited in the hair cortex to produce long-lasting color. Booster dyes are sometimes added to commercial formulations to tune the resulting hair color. Depending on the composition of the precursors and their relative amounts in a dye kit, different hair colors can be achieved.

This study seeks to examine, characterize, and identify dyes extracted from human hair via instrumental analysis for the purpose of determining common origin. UHPLC-ESI-TOF/MS was used to identify the components of permanent hair dyes. A UHPLC separation of dyed hair sample extracts was indicative of a small collection of predicted oxidation dye products, though with very low intensities. Dyes formed from oxidation reactions are more difficult to extract and detect than additive or booster dyes found in the same dye kit. Dye signals can vary in intensity among different permanent dye formulations of similar colors, even when they contain similar precursor molecules. Extraction method studies determined the heated methanol extraction procedure provided better extraction of oxidation dyes than a sodium hydroxide hair digestion. It was also determined that there is no direct correlation between the length of a hair sample and the intensity of detected dyes, limiting the forensic value of quantitative dye measurements.

Hair, Liquid Chromatography, Mass Spectrometry

B160 Analysis of Change in Nitrite-to-Nitrate Ratios in Gunshot Residue Over Time Using Ion Pairing High-Performance Liquid Chromatography (HPLC)

Anusha Rankoth, 100 College Drive, Allentown, PA 18104; Marianne E. Staretz, PhD, Cedar Crest College, Dept of Chemical & Physical Sc, 100 College Drive, Allentown, PA 18104; Peter J. Diaczuk, BS, 445 W 59th Street, New York, NY 10019-2925; Thomas H. Pritchett, MS, 100 College Drive, Allentown, PA 18104; and Elana Conant, MS, Cedar Crest College, 100 College Drive, Miller #10, Allentown, PA 18104*

After attending this presentation, attendees will be familiar with an ion-pairing HPLC method for measuring nitrate and nitrite that can be a useful analytical tool in Gunshot Residue (GSR) analysis.

This presentation will impact the forensic science community by introducing an HPLC method that can measure the levels of nitrate and nitrite in GSR in the barrel of a firearm. This method has the potential to determine the time frame in which a firearm was last discharged.

There is a high level of nitrites and nitrates present in GSR due to the high nitrogen content of the propellant used in smokeless powder cartridges. After discharge, these components are left behind within and on the firearm. Given that nitrite oxidizes to nitrate, it is feasible to assume that the nitrate-to-nitrite ratios within the gun barrel residue will change with time. Therefore, a gun that was fired several days prior might be expected to have a lower nitrite-to-nitrate ratio than a gun that was fired more recently; however, because different ammunition manufacturers use different ingredients when constructing their cartridges, these ratios may differ among various ammunition types.

This study has developed an HPLC method which can be utilized to measure the nitrite-to-nitrate ratios within the barrel of a firearm after it has been discharged and can determine if there is any change in this ratio over time that may prove useful for estimating the time frame in which this discharge occurred. This method consisted of an ion-pairing system using the ion-pairing agent tetrabutylammoniumhydrogen sulfate. Successful separation of the target analytes is possible in less than five minutes. The HPLC data was collected using an Agilent® 1100 HPLC system. The system utilized a diode array detector and absorbance was monitored at 205nm. Separation was accomplished using a 100mm x 2.1mm x 2.6µm Kinetex® C_{18} column. The mobile phase consisted of 95% 10mM tetrabutylammonium hydrogen sulfate in a 2.0mM sodium phosphate buffer adjusted to a pH of 8.4 and 5% HPLC-grade acetonitrile. Quantitation of nitrite-to-nitrate ratios was completed by analyzing the respective peak area via Agilent® Chemstation. Sample injections of 20µL were made using an autosampler at a flow rate of 0.20ml/min.

Gunpowder (smokeless powder) was burned in the open and samples were collected at various time intervals using Absorbond® polyester swabs dampened with HPLC-grade water. The swabs were then placed into 2ml of mobile phase. Extraction was carried out for 10-15 minutes followed by filtration using 0.2µm syringe filters. Filtered samples were placed into autosampler vials for analysis.

The retention time of nitrite present in GSR using this method is 2.4-2.5 minutes. The retention time for nitrate in GSR samples is 3.9-4.0 minutes. Starting ratios of nitrite to nitrate at time zero range from 1 to 0.92. After 24 hours, the ratios decreased to a range of 0.45 to 0.49. After an additional 24 hours, the ratios decreased again to approximately 0.27. These results indicate that nitrite-to-nitrate ratios decrease approximately 50% over a 24-hour period. With this type of data, it may be possible to construct a calibration curve which will be an important tool in determining the approximate time a firearm was last discharged.

Separation of nitrite and nitrate using this isocratic ion pairing system proved to be swift and reliable. The method was applied to the analysis of gunpowder residue. The oxidation of nitrite to nitrate in the sample was confirmed using this method. It was also confirmed that the nitrite/nitrate ratios decreased over time. This method will be utilized to analyze GSR in the barrel of a firearm at various time intervals after firing. These results will also be presented.

Gunshot Residue, HPLC, Ion Pairing Chromatography

B161 Evaluation and Validation of a Model to Quantify the Weight of Fingerprint Evidence

Henry J. Swofford, MSFS, 4930 N 31st Street, Forest Park, GA 30297; Anthony Koertner, 4930 N 31st Street, Forest Park, GA 30297; and Michael J. Salyards, PhD, 45 High Street, Sharpsburg, GA 30277*

The goal of this presentation is to introduce attendees to a novel method for quantifying the weight of fingerprint evidence, which has been developed and is currently undergoing validation by the United States Army Criminal Investigation Laboratory. After attending this presentation, attendees will have a better understanding of the difficulties with supporting claims of single-source attribution (e.g., "individualization") and be introduced to an alternative framework implemented within the Department of Defense.

This presentation will impact the forensic science community by helping attendees better understand the mathematical concepts by which this method was developed, the results of preliminary evaluation data against mated and non-mated fingerprints obtained from a database of several million fingerprints, and the on-going validation efforts to facilitate the transition of this technology into practice. This presentation will explore the evolution of fingerprint testimony, highlight potential issues with the current reporting paradigm, and recommend an alternative reporting framework to ensure fingerprint results are reported in an epistemologically compatible and more scientifically defensible manner.

For more than 100 years, fingerprint evidence has been used as a valuable tool for the criminal justice system. Relying on the generalized premise of "uniqueness," the forensic and legal communities have regarded fingerprint evidence as nearly infallible, having the capacity to "individualize" the source of a fingerprint impression to a single individual. While the uniqueness of a complete record of friction ridge skin detail is generally undisputed, the extension of that premise to partial and degraded impressions has become a central issue of debate. As a result, the traditional paradigm of reporting latent fingerprint conclusions with absolute certainty to a single source has been challenged. The underlying basis for the challenge pertains to the mathematical logic applied and the manner in which the evidence is articulated. By recognizing the subtle yet non-trivial differences in the mathematical logic, the fingerprint community may consider an alternative framework to report fingerprint evidence to ensure the certainties are not over- or understated. This presentation will discuss the logic largely subscribed to by the fingerprint community along with the underlying basis to why it is the focus of challenge, present an alternative framework for the community to consider adopting which is epistemologically more compatible and defensible, and discuss how this transition was achieved within the Department of Defense without minimizing the value of fingerprint evidence.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the United States Department of the Army or United States Department of Defense.

Fingerprints, Likelihood, Statistics

B162 I Know It When I See It — Is Complexity the Key to Creating a Workable Documentation Policy for the Pattern Evidence Disciplines?

Heidi Eldridge, MS, RTI International, 3040 E Cornwallis Road, RTP, NC 27709*

After attending this presentation, attendees will be able to articulate the main arguments in favor of the creation of policies requiring contemporaneous documentation of the basis for conclusions in the pattern evidence disciplines. Preliminary data will illustrate how consensus complexity determinations can be used to design an operationally reasonable policy, using latent prints as a model system. Attendees will also be able to describe the challenges in defining complexity in highly interpretive disciplines. Finally, attendees will be equipped with practical suggestions on how to design and implement such policies in their own laboratories.

This presentation will impact the forensic science community by frankly discussing the need for documentation in the pattern comparison disciplines, then by providing a roadmap for designing and implementing a policy that meets those needs without being overly cumbersome operationally. This presentation will present data from multiple exercises to achieve this goal and provide suggestions for moving forward in light of these data.

The pattern evidence comparison disciplines (e.g., latent prints, handwriting, firearms and tool marks, and footwear and tire marks) are highly interpretive in nature. When there is a high level of human interpretation involved, variability inevitably follows. This variability can lead to a host of issues, notably inconsistency in conclusions and difficulty in demonstrating reliability. Without documentation of the basis for a conclusion, it is difficult to resolve these issues.

If two examiners disagree on a conclusion, they have no way to articulate the reasons behind their differing conclusions without documentation as to how they reached those conclusions. If an error is discovered (typically months or years after the fact), there is no way to perform a root cause analysis if there was no documentation made of how the incorrect conclusion was reached. Without documentation, there is no way to assess the validity of a conclusion, whether that assessment is being made by a reviewer, an opposing expert, or a trier of fact.

In trying to develop a documentation policy, the first instinct of many laboratories is to base the policy on the complexity of the unknown image — a poor quality image requires more documentation than a pristine one; however, there are challenges in taking this approach. “Complex” is a term that has not been well-defined and the complexity threshold itself is subject to a level of variation between practitioners.

Using latent prints as a model system, exercises were created and executed in order to identify and isolate the components of complexity and create a process by which consensus on image complexity could be reached, such that a documentation policy could be built around it. This study presents the results of that effort, along with a description of the process, a roadmap for repeating the process in any laboratory, and suggestions for practical documentation policies that will achieve the goals of documentation without being too operationally cumbersome.

The National Institute of Justice (NIJ) Forensic Technology Center of Excellence (FTCoE) is committed to improving the practice of forensic science and strengthening its impact to agencies dedicated to combating crime. This FTCoE presentation recognizes the importance of balancing the implementation of best practices with recognizing the operational needs of a functional forensic science laboratory.

Documentation, Pattern Evidence, SOPs

B163 A Bibliometric Review of the Impact of the National Academy of Sciences (NAS) Report on the Friction Ridge Discipline

Maria A. Roberts, 2501 Investigation Parkway, Quantico, VA 22135; Kathryn B. Knorr, MS, 2501 Investigation Parkway, Quantico, VA 22135; and Kyle Tom, MS, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, attendees will better understand the transition friction ridge research made after the National Research Council on the 2009 NAS Report, Strengthening Forensic Science in the United States: A Path Forward, was published.

This presentation will impact the forensic science community by comparing research trends in the friction ridge discipline both pre-NAS Report and post-NAS Report. A bibliometric review will be contrasted against an informal review of the literature considered important by Subject Matter Experts (SMEs) in the friction ridge discipline.

The impact of the NAS Report on friction ridge literature was measured in a bibliometric review by using an online literature indexing tool, Web of Science™, and focusing on the five years before and after the NAS Report. This bibliometric review showed an increase in the number of friction ridge articles indexed in Web of Science™ from an average of 22.6 articles per year between 2005 and 2009 and an average of 41 articles per year between 2010 and 2014. The predominant funding agencies also changed: the National Institute of Justice, Federal Bureau of Investigation, and the Home Office (United Kingdom) became the top three contributors post-NAS Report, but none of them were in the top three pre-NAS reports. Four of the five most prolific authors remained in the top five between both time frames, and all five countries remained in the top five.

A search of common terms associated with fingerprints produced an overwhelming amount of unassociated articles since the term "fingerprint" is used outside the friction ridge discipline. Web of Science™ lacks a specific forensic science category, so several categories were selected to focus on the appropriate articles; however, this lack of specificity required an additional manual screening process to remove articles that were visibly not associated with the friction ridge discipline. Sensitivity of a bibliometric review is reduced due to limiting the search on specific categories, as well as not containing journals that are not indexed.

Using bibliometric tools to review literature has its limitations, such as relying heavily on key words, a lack of a specific forensic science category, and partial lists of indexed journals. The methods, though, are objective, and the results can be quantified. This broad, calculated, and repeatable approach of a bibliometric review was contrasted to a more concise, influential, but subjective list of articles that was organically produced by the friction ridge discipline in response to the NAS Report.

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NAS Report, Bibliometric Review, Friction Ridge

B164 Fingerprint Aging Mechanism Determination Through Electrochemistry

Roberto Rosa, PhD, University of Modena and Reggio Emilia, Via Pietro Vivarelli 10, Modena 41125, ITALY; Roberto Giovanardi, PhD, University of Modena and Reggio Emilia, Dept of Engineering Enzo Ferrari, Via Pietro Vivarelli 10, Modena 41125, ITALY; Andrea Bozza, MSc, University of Modena and Reggio Emilia, Dept of Engineering Enzo Ferrari, Via Pietro Vivarelli 10, Modena 41125, ITALY; Paolo Veronesi, PhD, Dept Materials and Environmental Engineering, via Vignolese 905, Modena 41125, ITALY; and Cristina Leonelli, PhD, Dept Materials and Environmental Engineering, via Vignolese 905, Modena 41125, ITALY*

After attending this presentation, attendees will understand the great potentialities offered by electrochemical techniques in the fascinating and highly challenging research field of fingerprint age determination. Currently, a reliable, quantitative, and reproducible technique for determining the age of fingerprints found at the crime scene is still lacking, although it is considered one of the major challenges for future forensic science innovation.[1,2]

This presentation will impact the forensic science community by demonstrating a quantitative determination of the fingerprint aging mechanism through Electrochemical Impedance Spectroscopy (EIS) measurements. Prior to a reliable method for fingerprint age determination being effectively developed, a deeper understanding of the aging mechanism is necessary as research efforts must be refocused on the fundamental understanding of the fingerprint itself.[3]

This study seeks to accurately investigate the fingerprint aging mechanism on selected metallic substrates, namely AISI316L stainless steel, aluminum (6082), and brass. The chemical as well as the physical modifications which a fingerprint undergoes over time will surely affect the electrochemical response of the system, constituted by the print residue and the substrate on which it has been deposited.

As recently recommended by the International Fingerprint Research Group (IFRG), natural "ungroomed" marks were tested in order to evaluate the proposed technique for actual scenarios.[4] A three-electrode cell was employed for the EIS measurements, using the metallic substrate bearing the fingerprint as the working electrode, a platinum foil as the counter electrode, and $Ag/AgCl/KCl_{sat}$ as the reference electrode. The impedance spectra were acquired in the 100kHz÷100mHz frequency range (ω), applying a sinusoidal potential wave (0V bias, 10mVrms of amplitude). In this way fingerprints were monitored over a time period of 45 days.

The equivalent circuit used to fit the experimental impedance data collected at different aging times was a Randle's one R(QR), consisting of an ohmic resistance, mainly ascribed to electrolyte (i.e., 0.2 M Na2SO4) solution, in series with the parallel combination of the double-layer capacitance (C_{dl}) and the charge transfer resistance (R_{ct}) of the reaction activated during the slight polarization applied to the substrate. The double-layer arrangement of the systems investigated in this study differs from an ideal capacitor, thus a Constant Phase Element (CPE) whose impedance contribution is reported in Equation 1 has been used to replace C_{dl}.

Equation 1: $Z(w)=Y_0(jw)^{-n}$. Y_0 represents a constant with dimension $F\times s^{n-1}$, ω is the applied frequency, while j=Ö-1and $0<n<1$. The EIS results showed that the fingerprint mainly affects the capacitance of the double layer formed at the metal substrate/electrolyte interface: the fingerprint aging process has, as its main effect, a decrease of the active area of the metal substrate (due to the spreading of the fingerprint on the metal surface), a phenomenon which leads to a decrease of the capacitance of the electric double layer, directly correlated with the ageing process, through a 3-parameters exponential decay equation of the type in Equation 2.

Equation 2: $y=y_0+ae^{-bx}$. Similarly to what observed for the Y_0, the exponent n reported in Equation 2 also undergoes an exponential decay during the aging of the fingerprint. This latter behavior can be correlated to a variation in the roughness of the fingerprint-substrate system, in accordance to literature data.[5] On the contrary, the R_{ct} value does not significantly change during the aging process: this result suggests that the only faradaic reaction activated during the slight polarization applied to the substrate is the oxidation of the metallic substrate (hypothesis confirmed by the high R_{ct} values obtained, which are consistent with the passive behavior of the metals used when exposed to the selected electrolyte).

In conclusion, EIS allowed this study, for the first time, to quantitatively clarify the aging mechanism of fingerprints on metallic substrates, smoothing the way for reliable fingerprint age determination studies and for considering the possibility of easily coupling EIS to further electrochemical-based techniques (this latter aspect being the main focus of current research).

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Fingerprints, Aging Mechanism, Electrochemistry

B165 Performance and Ricochet Characteristics of Frangible Ammunition

Peter J. Diaczuk, BS, 445 W 59th Street, New York, NY 10019-2925; Jack Hietpas, PhD, FBI-ORISE, 2501 Investigation Parkway, Quantico, VA 22135; and Xiao Shan Law, BS, 1937 W 6th Street, Apt 2R, Brooklyn, NY 11223*

After attending this presentation, attendees will understand the difference between conventional ammunition and frangible ammunition. Attendees will also become familiar with several types of frangible ammunition, whether these types of frangible bullets acquire individual markings after being fired, and how some break apart into fragments that bear little resemblance to a bullet if found at a shooting scene.

This presentation will impact the forensic science community by exploring the components of the frangible ammunition obtained for the study, how they behave upon impact, and whether fired bullets are amenable to comparison microscopy.

Frangible ammunition is constructed in a similar fashion to conventional ammunition regarding the cartridge case, the primer, and the propellant, but that's where the similarity ends. Conventional bullets, often consisting of a dense lead core either fully or partially enclosed with a harder copper jacket, or even unjacketed, can remain intact after a ricochet or after perforating a barrier. Ammunition that is manufactured with a frangible bullet is designed to minimize the dangers from ricochet or unwanted barrier perforation by using a bullet that is constructed to break up or fragment upon impact with hard unyielding substrates. The energy of these smaller post-impact fragments or powder is so small that they cannot travel very far from the initial impact site. To perform this way, these bullets are made of various formulations of powdered metals held together by adhesives, resins, or polymer materials. In contrast to the formulations that are bonded together, at least one manufacturer has developed a line of frangible ammunition that instead incorporates a jacket to encase the frangible core. Because of this novel design, the manufacturer claims that their bullets will also behave on soft organic targets as they behave on hard unyielding materials (i.e., by breaking up into small pieces). The properties of several brands of frangible ammunition were tested in scenarios that could be encountered in a shooting scene.

Several types of frangible ammunition that included a variety of construction methods and constituents were analyzed. Cartridges from different manufacturers were disassembled and the bullets were cross-sectioned to gain an understanding of their construction. Stereomicroscopy was used to assess how the bullet was made and its constituents; then Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS) was used to identify the elements present. The impact dynamics of the various frangible bullets tested were compared to traditional Full Metal-Jacketed (FMJ) bullets with unyielding material (steel plate) and yielding materials (wet sponge and sheet metal). The frangible bullets were also assessed based on their ability to accept individual markings (i.e., stria) from passage down the barrel of the firearm. High-speed photography was used to gain an understanding of the bullet's performance characteristics on the aforementioned yielding and unyielding materials.

Recovered bullet or jacket fragments were examined microscopically to determine if stria were present from the barrel's rifling and, if so, whether they were useful for comparison purposes. Frangible bullets that were encased within a traditional jacket did retain stria from the barrel and were able to be compared successfully to other test-fired bullets from the same firearm. Those that were not jacketed and were made of composites of powdered metal and adhesive only had class characteristics imparted from the rifling of the firearm's barrel. While the conventional FMJ bullets remained intact after 15-degree incident-angle impact with steel plate, most frangible bullets broke up into fragments upon impact with the same steel plate. Some of these post-impact bullet fragments were still relatively large and retained enough energy to pose a danger at close range.

Frangible, Bullet, Comparison Microscopy

B166 Development of a New Standard Bullet for Ballistic Quality Control

Thomas B. Renegar, BS, NIST, 100 Bureau Drive, MS 8212, Gaithersburg, MD 20899; Xiaoyu A. Zheng, MS, NIST, 100 Bureau Drive, MS 8212, Gaithersburg, MD 20899; Robert M. Thompson, BS, NIST, Special Programs Office-Forensic Sciences, 100 Bureau Drive, MS 8102, Gaithersburg, MD 20899; Theodore V. Vorburger, PhD, NIST, 100 Bureau Drive, MS 8212, Gaithersburg, MD 20899; Junfeng J. Song, MS, NIST, 100 Bureau Drive, MS 8212, Gaithersburg, MD 20899; Johannes A. Soons, PhD, NIST, 100 Bureau Drive, MS 8223, Gaithersburg, MD 20899; and James H. Yen, PhD, NIST, Statisical Engineering Division, 100 Bureau Drive, MS 8980, Gaithersburg, MD 20878-8980*

After attending this presentation, attendees will understand the importance of the National Institute of Standards and Technology (NIST) Standard Bullet and how it relates to ballistic measurement quality control and traceability. Attendees will also understand polymer replication and metal-coating techniques.

This presentation will impact the forensic science community by demonstrating a method of ensuring quality control for ballistic measurements. This is important in how it relates to standardization organizations and laboratory accreditation.

This presentation describes the development of the next generation Standard Reference Material (SRM) Standard Bullet. The original Standard Bullet (SRM 2460) was developed by NIST to provide a standardized physical artifact for forensic laboratories to demonstrate quality control and measurement traceability.

Forensic laboratories that perform ballistic comparisons have come under scrutiny in recent years to ensure that sound scientific methods and quality control are followed in their measurements and analysis. For ballistics identifications, it is important that measurement equipment is operating properly and appropriate measurement practices are followed. The original NIST SRM Standard Bullet, developed more than ten years ago, has been an invaluable tool for forensic examiners.[1] By using the Standard Bullet, examiners ensure their measurements, comparisons, and analysis of bullet evidence are done properly. Examiners are also able to show measurement traceability, which allows measurement comparisons to be performed with other laboratories.

The original NIST SRM 2460 Standard Bullet is no longer available. A suitable replacement is needed that will meet the needs of forensic laboratories. The replacement Standard Bullet must meet several requirements: (1) it must have similar topography to that of the original Standard Bullet; (2) surface features (striations) must be consistent from one unit to the next so comparisons between laboratories can be performed; (3) it must be cost effective; and, (4) it must be durable for laboratory use. The original Standard Bullet was manufactured using a diamond turning machining process, which was time consuming and expensive. Therefore, an alternative manufacturing process was developed.

Polymer replications were produced from original master bullets using silicone molding and polymer casting techniques. Originally developed by the Bundeskriminalamt (BKA) in Germany and improved by NIST, a suitable manufacturing process was developed.[2] Several challenges that were faced will be discussed, including removal of micro-bubbles from the silicone and polyurethane mixtures. This is crucial to producing clean replications free of bubbles and other contaminations. Durability of the bullets is also important for laboratory use. Techniques used to harden the surface of the polymer replicas, including gold coating, will be presented. The manufacturing process for producing more than 100 bullets will also be discussed as well as the measurements/analysis performed to ensure the quality of all bullets produced.

Going forward, the new Standard Bullet will be a key part of ensuring bullet comparisons are performed using quality-control practices. This will be an important part of laboratory accreditation. New documentary standards that are currently being developed by the Organization of Scientific Area Committees (OSAC) will undoubtedly require laboratories to follow quality-control practices and demonstrate measurement traceability. The new Standard Bullet will help fulfill these requirements.

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2. Koch A., Katterwe J. Castings of Complex Stereometric Samples for Proficiency Tests in Firearm and Tool Mark Examinations, *AFTE Journal*, vol. 39 (4), 2007

Standard Bullet, Polymer Replication, Quality Control

B167 Analyzing a Firearms Proficiency Test Using the Congruent Matching Cells (CMC) Method of Computer-Aided Topography Comparisons

Daniel Ott, PhD, NIST, 100 Bureau Drive, Mail Stop 8212, Gaithersburg, MD 20899; Robert M. Thompson, BS, NIST, Special Programs Office-Forensic Sciences, 100 Bureau Drive, MS 8102, Gaithersburg, MD 20899; and Junfeng J. Song, MS, NIST, 100 Bureau Drive, MS 8212, Gaithersburg, MD 20899*

After attending this presentation, attendees will understand how computer algorithms can be used as a powerful tool for firearms examiners to quickly identify potentially matching regions on a surface.

This presentation will impact the forensic science community by addressing concerns about the scientific method in forensic science through the use of quantitative surface comparison algorithms and drawing connections to comparisons made by human examiners.

The underlying technology in this presentation is a computer algorithm developed at the National Institute of Standards and Technology (NIST) to compare impressed tool marks on ballistics evidence in an impartial, automatic, and quantifiable way. The algorithm is called the CMC method, which is based on the discretization of a surface into cells that are compared individually with another surface. The registration location of each cell is found which corresponds to the location of highest physically similarity on the other surface. Cells registered in the same relative orientation on the second surface are considered congruent and the number of congruent matching cells constitutes a similarity score. The CMC method has been effectively used to analyze select sets of data containing known matching and known non-matching tool mark pairs.[1] One of the key advantages of this method is that it is effective in isolating valid areas that contain unique surface topography from invalid areas without unique surface topography. In a way, this is similar to the human examiner's ability to disregard an area on a surface that is damaged, has pre-fired surface features, or does not contain useful features for correlation. Therefore, a direct comparison of the regions used to justify identifications can be made. This feature also allows the CMC method to be used as a tool to help a trained examiner quickly identify potentially matching regions for further scrutiny.

In this experiment, the CMC method was applied to a firearms comparison proficiency test administered by Collaborative Testing Services (CTS). The results of this proficiency test, along with comments by test takers, are provided by CTS. Therefore, the computer comparison algorithm can be directly compared with known data and known results from human examiners. The CTS test set was also analyzed under a comparison microscope. The features that were identified using the comparison microscope will be presented along with the areas that were identified as contributing to a match using the CMC method. These results will be supplemented with results and comments published by CTS regarding this test. A summary of the various evaluations shows that certain features are more easily identified using computer algorithms, but a holistic approach which utilizes the examiner's knowledge of firearm mechanics and class, sub-class, and individual characteristics is necessary to make informed ballistic evidence comparisons.

By identifying features that examiners and computers are able to effectively detect, a better understanding of the strengths of each type of examination technique can be reached. By combining the respective strengths of human examiners and computer algorithms, it is possible to achieve more robust and efficient surface comparisons. The primary goal is to speed up work flow by providing a tool to identify key features that could potentially match and strengthen an examiner's ability to justify and quantify their conclusions. This presentation demonstrates how the CMC method is able to achieve these goals in order to address recent concerns about the scientific method in forensic science.

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Ballistics Comparisons, Proficiency Tests, CMC Method

B168 Modeling Firearm Tool Mark Persistence Through Objective Surface Metrology and Analysis

Xiaoyu A. Zheng, MS, NIST, 100 Bureau Drive, MS 8212, Gaithersburg, MD 20899; Johannes A. Soons, PhD, NIST, 100 Bureau Drive, MS 8223, Gaithersburg, MD 20899; Robert M. Thompson, BS, NIST, Special Programs Office-Forensic Sciences, 100 Bureau Drive, MS 8102, Gaithersburg, MD 20899; and Wei Chu, NIST, 100 Bureau Drive, MS 8212, Gaithersburg, MD 20899*

After attending this presentation, attendees will better understand how firearm tool marks persist over many firings. Attendees will also learn the basics of 3D measurements and analysis using the cross-correlation function as well as standard surface texture parameters.

This presentation will impact the forensic science community by providing objective support for the reproducibility of firearm tool marks over many firings. This research builds upon previous research in which examiners were able to visually identify the first test fire against the rest of the persistence test fires. This presentation will also provide quantitative analysis of how the marking surface is changing over time.

The study objective is to quantify the microscopic changes to the marking surfaces of a firearm (land, breech face, and firing pin) and how these changes affect the reproducibility of the generated tool marks on bullets and cartridge cases. The 2008 Ballistics Imaging report stated, "The validity of the fundamental assumptions of uniqueness and reproducibility of firearms-related tool marks has not yet been fully demonstrated."[1] The report also state, "A designed program of experiments covering a full range of sources of variability is important to test the fundamental assumptions, as well as to better document phenomena like "settle-in" effects."[1] Previous research has been conducted to evaluate the level of persistence seen on firearm tool marks using visual comparisons of test fires throughout the lifetime of the firearm.[2,3] These studies have concluded that examiners are able to visually identify Test Fire 1 with the rest of the persistence sets. They also noticed wear over time and slight changes to the tool marks created.

The current research seeks to provide more objective measurement and analysis to quantify how the tool marks are changing over time. The degree of similarity between bullets and cartridge cases are evaluated using the maximum value of the normalized Cross Correlation Function (CCF) or Areal Cross Correlation Function (ACCF). To quantify the physical changes of the tool marks, standard surface texture parameters found in the American Society of Mechanical Engineers (ASME) B46.1-2009 are used.[4] These include arithmetic mean deviation of the assessed profile/surface (Ra, Sa), root mean square deviation of the assessed profile/surface (Rq, Sq), maximum profile peak height (Rp, Sp), maximum profile valley depth (Rv, Sv), and total height of the profile (Rt, St). The test fires used in this research were generated by the following groups: (1) Alameda County Sheriff's Office Crime Lab. Two thousand bullets and cartridge cases fired from a new Ruger® P89 pistol. The first ten, then every 25th, test fire was collected; (2) Indiana State Police Laboratory. Ten thousand bullets and cartridge cases fired from three new Beretta® 96G pistols. First three, then three more test fires at 500-round intervals were collected.

Measurements were taken with a disc-scanning confocal microscope which generates 3D topographic data. A 10X objective with 0.3NA was used for breechface measurements and a 20X objective with 0.6NA was used for firing pin and bullet measurements. Standard Gaussian filters were applied to extract the pertinent individual tool marks used for identifications. Several experiments were conducted to show the trend in CCF/ACCF and surface texture parameters over the test fire sequences. This research helps to provide more objective analysis of firearm persistence as well as evaluate the reproducibility of tool marks from a firearm.

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3. Gouwe J., Hamby J., Norris S., Comparison of 10,000 Consecutively Fired Cartridge Cases from a Model 22 Glock .40 S&W Caliber Semiautomatic Pistol. *AFTE Journal*, 2008, Vol 40, Number 1 (Winter), Page 57 thru 63
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Firearm Tool Marks, Persistence, Surface Metrology

B169 Proposed Congruent Match Cross-Section (CMX) Method for Ballistics Identification of Firing Pin Impressions

Junfeng J. Song, MS, NIST, 100 Bureau Drive, MS 8212, Gaithersburg, MD 20899; Mingsi Tong, PhD, NIST, 100 Bureau Drive, MS 8212, Gaithersburg, MD 20899; Hao M. Zhang, PhD, NIST, 100 Bureau Drive, MS 8212, Gaithersburg, MD 20899; Wei Chu, NIST, 100 Bureau Drive, MS 8212, Gaithersburg, MD 20899; and Robert M. Thompson, BS*, NIST, Special Programs Office-Forensic Sciences, 100 Bureau Drive, MS 8102, Gaithersburg, MD 20899*

After attending this presentation, attendees will understand how to use a new method called the CMX Method developed at the National Institute of Standards and Technology (NIST) for ballistics identification of firing pin impressions.

This presentation will impact the forensic science community by providing a new method — the CMX Method — for fast and accurate ballistics identification of firing pin images.

Reporting an error rate for forensic evidence identification is a fundamental challenge in forensic science.[1] It is a national priority to establish a scientific procedure for quantitative error rate reports to support firearm and impression identifications in court proceedings.[2] The Congruent Matching Cells (CMC) method was recently invented at the NIST for accurate image-related forensic evidence identification and error rate estimation.[3,4] The CMC method is based on the principle of discretization — it divides the entire image into small correlation regions and uses multiple identification parameters for accurate forensic evidence identification. This enables the estimation of error rates and the Likelihood Ratio (LR) based on statistical analysis of the total number of correlation cells, the number of qualified CMC cell pairs, and the statistical distribution of the four identification parameters.

Validation tests of the CMC method for correlation of breechface impressions have recently been completed using 40 cartridge cases fired with consecutively manufactured pistol slides.[5] These tests include 717 Known-Non-Matching (KNM) and 63 Known-Matching (KM) image correlations. The results do not produce any false positive or false negative identifications and hence provide strong initial support for the effectiveness of the CMC method for correlation of the breechface impressions. An approach for calculating error rates has also been developed using the CMC method.[4]

Firing pin impressions on cartridge cases are an important part of firearms evidence identifications; however, in comparison to the CMC correlation for breechface impressions with millimeter-sized correlation areas, there is only a limited correlation area (sub-millimeter) on the firing pin impressions. Furthermore, the concave shape of firing pin impressions makes it difficult for automatic correlations. A new method and related algorithm, CMX, are proposed for correlation of firing pin impressions. Each firing pin impression is sliced into layers; their circular cross-sections are converted into linear profiles by the polar coordinate transformation. The areal spline filter is used for extracting the high-frequency micro-features, or the individual characteristics, for accurate correlation.[6-8] Three identification parameters are proposed for determining whether these pair-wise firing pin impressions are fired from the same firearm. The Cross-Correlation Function (CCF) is used for quantifying similarity of the pair-wise profiles which represent the two correlated firing pin images. The registration phase angle θ is another important identification parameter: if the correlated cartridge pair is fired from the same firearm, there would be nearly a common phase registration angle between the set of profiles of the reference firing pin impression and those of the correlated firing pin impression. The vertical shift distance of the slice location h, combined with horizontal shift distance of the phase angle θ, are used for determining the congruency of the pair-wise correlated profiles. When these parameter values and their statistical distributions are collected for analysis, the CMX number is derived as a key parameter for a conclusive identification of exclusion.

The CMX method and the proposed identification algorithm were validated by 780 pair-wise firing pin topography images of 40 cartridge cases of three brands, which were fired from ten firearms from three different manufacturers. This includes 60 KM and 720 KNM image pairs. All of these topography image pairs are correctly identified based on the optimized parameters. There is a clear separation between the CMX distribution of the KM and KNM image pairs: for the 720 KNM image pairs, the CMXs are distributed from 0 to 14; for the 60 KM image pairs, CMXs are distributed from 20 to 49.[9] Although there is only a limited data size collected from three brands of 40 cartridges fired by ten firearms from three manufacturers in this study, it has demonstrated the possibility of using the proposed CMX method for correlations of large data sets of firing pin images fired from different firearms.

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Forensic Science, Firearm Identification, Congruent Match Cross-Section

B170 Imparting a Meaningful Application of Statistics to Forensic Scientists

Stephen L. Morgan, PhD, University of South Carolina, Dept of Chemistry & Biochemistry, 631 Sumter Street, Columbia, SC 29208*

After attending this presentation, attendees will understand that statistical education is fundamental to the proper analysis of scientific data, including that generated by forensic laboratories. Understanding the meaning and appropriate application of statistics is vital if conclusions reached are to have validity. This presentation will discuss the examples of inappropriate and appropriate statistics that should be considered essential knowledge for the practicing forensic scientist.

This presentation will impact the forensic science community by discussing approaches to addressing inadequate applications and interpretations of statistics in forensic science by providing examples of appropriate usage and highlighting selected pitfalls in statistical methodology and interpretation.

The book, How to Lie with Statistics, states that "anything smacking of the medical profession" (or backed by scientific laboratories) is worthy of trust.[1] H. G. Wells once said, "Statistical thinking will one day be as necessary for efficient citizenship as the ability to read and write." The assault on inappropriate statistical thinking in forensics began in 2004 with the National Academy of Sciences (NAS) Report on bullet lead comparisons.[2] The following 2009 National Academy of Sciences (NAS) Report, Strengthening Forensic Science in the United States – A Path Forward, emphasized validation of forensic measurements and estimation of uncertainty of measurements to understand sources of bias/error in forensic science.[3] Just substitute "forensic scientists" for "efficient citizenship" in the Wells quote. The day is now — trust is no longer unconditional.

Education in applicable statistics is a first step. Accreditation Standards of the American Academy of Forensic Sciences (AAFS) Forensic Science Education Programs require "…at least one course in statistics (three semester hours)." There is no mention of statistics applicable to forensic practice, but budding forensic scientists have exposure to method validation in the suggested instrumental analysis course. What about a meaningful discussion of reporting confidence intervals instead of p-values, of differences between a p-value, a size effect, or a likelihood ratio; or of the fact that at a limit of detection defined by the concentration equivalent to a signal that is three standard deviations of the blank measurement higher than the average blank signal, the false positive detection rate is 50%. Houck was perhaps concerned with the "tyranny of numbers" when he wrote, "This expectation (to apply statistics) is fraught with pitfalls that could adversely affect the accuracy of evidentiary reports presented in court. The foundational data upon which trace evidence statistics might be based differ radically from those used in DNA statistical calculations. If statistics are to be applied to trace evidence, they must be applied in a way appropriate to the discipline, unbiased in interpretation, and accessible to the trier of fact".[4] Reinhart believes the problem is poor statistical education.[5] Ioannidis makes the case that even established medical scientists have issues with positive predictive value (i.e., research producing false positive outcomes).[6]

Lack of knowledge of appropriate statistics in various disciplines is not uncommon. Medical students are required to take a course in statistics, yet medical residents apparently average less than 50% correct on questions concerning statistical tests used in medicine.[7] Most forensic validation documents are now citing appropriate statistical methodology; however, few show worked examples of appropriate data treatment for determining uncertainties or offer interpretations of the statistical outcomes. At this time, there is a need for statistical tools that are designed and fit-for-purpose in forensic application. How does the community get from here to where it should be is the question raised in this presentation.

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Statistics, Applicability, Interpretation

B171 Challenges for Implementing a New Paradigm in Fire Debris Analysis and Reporting

Mary R. Williams, MS, National Center for Forensic Science, PO Box 162367, Orlando, FL 32816-2367; and Michael E. Sigman, PhD, University of Central Florida, National Center for Forensic Science, PO Box 162367, Orlando, FL 32816*

After attending this presentation, attendees will understand the challenges of moving from categorical statements to statements of quantifiable evidentiary value in fire debris data interpretation.

This presentation will impact the forensic science community by demonstrating a methodology for assessing and presenting statements of quantifiable evidentiary value in fire debris analysis.

The American Society for Testing and Materials (ASTM) E1618-14 (Section 12) allows for reporting the presence or absence of ignitable liquid residue using a variety of terminology; however, "Note 5" stipulates that "[R]regardless of the choice of phrases used, there is no implied difference in the perceived level of confidence for a positive result."[1] The E1618-14 standard establishes subjectively determined dichotomous categorical statement(s) and explicitly rejects the concept of evidentiary value. This approach is not congruent with current trends in forensic science.

Many disciplines of forensic science have come under pressure to replace subjective methods and categorical conclusions with objective methods and/or statements that reflect statistical and quantifiable results. Popular ways to address this problem include the use of random match probabilities, likelihood ratios, and verbal equivalents to express the results in language that a lay jury can understand. Recent research in criminal justice and the social sciences have shed some light on the effectiveness of these three methods.[2,3] The approach that has been endorsed by the European Network of Forensic Institutes in their 2015 guideline on evaluative reporting involves the use of likelihood ratios in a Bayesian framework to express evidentiary value as a verbal scale which is tied to the likelihood ratio.[4] There are many challenges to implementing a likelihood ratio approach with verbal equivalents of evidentiary value in forensic fire debris analysis. This presentation will introduce the fundamental concepts of the approach and the benefits as well as the challenges that must be overcome to implement the approach. Topics addressed will include the need for specialized software, standard data sets, and accessible training.

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Fire Debris, Evidentiary Value, Bayesian Statistics

B172 An Overview of Different Approaches to Expressing Significance in Associative Forensic Reports

Christopher R. Bommarito, MS, Forensic Science Consultants, 127 W Grand River Avenue, Williamston, MI 48895*

After attending this presentation, attendees will better understand the varying methods used to convey significance in forensic reports involving comparison of trace evidence.

This presentation will impact the forensic science community by providing an overview of the importance of expressing significance in forensic comparisons, the different methods currently employed to express significance, and the advantages and disadvantages of each method.

In February 2009, the National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward was released*.[1] The Report expressed the need for the forensic community to "raise the standards for reporting and testifying" and identify in reports "the sources of uncertainty in the procedures and conclusions along with estimates of their scale (to indicate the level of confidence in the results)". This has increased the urgency among standard-setting organizations, such as the Scientific Working Group for Materials Analysis (SWGMAT), the European Network of Forensic Science Institutes (ENFSI), and the Organization of **Scientific Area Committees (**OSAC) to create standards by which analysts express significance in their reports.

Ideally, significance would be expressed with a frequency, as is done in DNA analysis. In most cases with manufactured materials, this is not possible due to both lack of information and the changing nature of the population of manufactured materials. Given these limitations inherent in expressing significance, several approaches have evolved to express significance. Many analysts have not changed their reporting from historical "could have originated from the same source" conclusions or have added statements to the conclusion to outline limitations of any associations made. While expressing limitations is an improvement over a simple "could have" conclusion, this approach does not offer context regarding the relative significance of the association.

There are longtime proponents of the use of an associative evidence scale to express various possible conclusions, strengths, and limitation to an association. This approach has been adopted by many forensic laboratories in the United States and abroad and has been the basis of attempts by some standard-setting organizations to form a consensus document on expressing significance in associative trace evidence. The evolution and practice of this approach will be outlined in this presentation. The associative scale has received criticism for being subjective and inherently unscientific.

A method that has gained support, primarily outside the United States is a Bayesian approach using likelihood ratios to express the significance of an association. The ENFSI has written a comprehensive standard supporting the use of likelihood ratios in trace evidence reports as well as examples on how such reporting should be utilized.[2] One concern over the use of likelihood ratios is that, while considered a valid statistical method, many of the frequency assumptions utilized are simple estimates and therefore also subjective. Labor-intensive frequency studies have been utilized in the calculation of likelihood ratios, but consensus has not been reached as to which frequencies to employ. Complicating matters is the necessity for these frequency studies to be repeated from geographical area to geographical area.[3] In addition, a recent study has shown that it is difficult for juries to understand the verbal conclusions assigned to describe likelihood ratios.[4]

In conclusion, this presentation will offer an overview of the current trends in expressing significance in associative trace evidence and demonstrate the benefits and limitations of each approach.

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Associative Scale, Significance, Likelihood Ratios

B173 Challenges in Developing Objective Interpretation Methods for Firearm and Tool Mark Examination

Robert M. Thompson, BS, NIST, Special Programs Office-Forensic Sciences, 100 Bureau Drive, MS 8102, Gaithersburg, MD 20899*

After attending this presentation, attendees will appreciate some of the major challenges in developing objective interpretation models in support of the subjective conclusions in firearm and tool mark comparison examinations.

This presentation will impact the forensic science community by exposing some of the scientific, technological, and training hurdles to be overcome before 2D and 3D tool mark surface comparisons are accepted in court.

Over the past few years, the broad scientific disciplines that make up the "Forensic Sciences" have experienced an accelerated interest in developing scientifically validated and rigorous methods to objectively measure examination results to augment the examiner's subject-based conclusion or opinion. The impression pattern disciplines currently are the greatest focus of such research due to the fact that examination results are fundamentally cognitively constructed by the examiner's observation skill, training, and experience. Firearm and tool mark examination and identification is a field in which objective measurement of the similarity of tool mark surfaces would be valuable to assist the examiner in supporting or refining the ultimate conclusion in examinations. This Research and Development (R&D) effort has been greatly assisted by increased resolution of digital photomicrography, instruments that measure 3D topographical micro-surface topography, and modern computational hardware and software; however, there are important challenges and obstacles that have to be addressed before a successful presentation of evidence, analysis, and expert opinion are delivered in a courtroom. This presentation will review what are believed to be the primary future challenges.

Technological: The forensic science profession is a small market compared to other commercial interests; the tool mark profession is even smaller. The instrumentation used in the nascent 2D imaging and 3D topographical comparison systems must be engineered by companies who have an interest in ultimately selling their systems in the future. The instrument manufacturers also must be convinced of the value in employing international standards in file transfer/sharing so that interoperability is baked into their technology from the onset. Additionally, each system brings performance strengths and weaknesses so it is probable that a number of objective comparison schemes must be tested and validated.

Measurement of Error and Confidence: Objective measurement of similarity necessarily must be paired with an objective conclusion of source. The conclusion may be based on an empirically derived threshold where identification of source is supported or based on the measured similarity quantity in relation to numerous known non-match comparisons where any similarity is due to chance. Both of these approaches require statistically valid databases of fired bullets and casings where the ground truth is known. Additionally, the databases must be adequately large and represent differences in caliber, ammunition materials, and firearm production types typical to those submitted to crime laboratories. For any determination of error rate, random match, confidence, or likelihood ratio, very large databases are warranted so that any of these results are scientifically valid.

Training: The examiners must be trained with the new technology so they are comfortable with foreign concepts and protocols such as objective measurement of tool mark similarity, how that is accomplished, the mathematical/statistical underpinnings of the comparison and the results, and how the statistical weight and measurement of confidence in a comparison is reported and best testified to a lay jury. Some thought and research on how to "model" accurate communication of these principles for testimony is required. Training the legal profession and judges in these "new" methods may be lacking and only conveyed in adversarial court settings in many jurisdictions. Having a robust and vigorously validated technology and method for casework is necessary for a successful adoption of the future tool mark comparison methods.

Objective Comparison, Firearm and Tool Mark, Similarity Measurement

B174 Mixture Interpretation and Statistics Town Hall Meeting

Kristy Kadash, PhD, Jefferson County Regional Crime Lab, 200 Jefferson County Parkway, Golden, CO 80401; Todd W. Bille, MS, Bureau of ATFE, National Laboratory Center, 6000 Ammendale Road, Ammendale, MD 20705; Charles H. Brenner, PhD*, 6801 Thornhill Drive, Oakland, CA 94611-1336; Michael D. Coble, PhD*, 100 Bureau Drive, MS 8312, Gaithersburg, MD 20899-8312; Norah Rudin, PhD*, 650 Castro Street, Ste 120-404, Mountain View, CA 94041; and Joel D. Sutton, MSFS*, 4930 N 31st Street, Forest Park, GA 30297-5205; Brad Jenkins, MS*, 700 N 5th Street, Richmond, VA 23219*

After attending this presentation, attendees will have a greater understanding of the impact that biological phenomena, amplification artifacts, and making assumptions can have on the conclusions that are drawn from DNA mixture profiles.

This presentation will impact the forensic science community by providing an open forum for discussing key factors in DNA profile interpretation.

The primary objective of this session is to share opinions and experiences regarding mixture interpretation. Forensic DNA interpretation is in a period of transition or, to use the theme of the 2016 meeting, a period of "transformation." It is clear that a "cookbook" approach to analyzing mixtures is not feasible and that a static set of rules cannot be applied to the interpretation of every DNA profile; however, it may be possible to generate a list of technological and biological phenomena for analysts to consider throughout the analysis and interpretation process. Likelihood Ratios (LR) are now considered to be the more appropriate statistical method for the majority of mixture conclusions. In addition, sophisticated modeling algorithms (probabilistic genotyping) are being employed to account for the behavior of DNA during Polymerase Chain Reaction (PCR) amplification. There is a great deal of uncertainty in how to apply probabilistic genotyping and LRs, particularly concerning what assumptions to make and what factors to incorporate for allele sharing, relatedness, stochastic events, PCR artifacts, and other casework scenarios. Furthermore, how should these assumptions and conclusions be documented, reported, and presented in court?

Rather than holding individual presentations on this topic, this Special Session of the Criminalistics Forensic Biology Scientific Session is intended to be an interactive discussion. This will not be a series of demonstrations of various software programs or theoretical explanations of the mathematical formulas. Instead, six panelists from diverse backgrounds (practitioners, researchers, mathematicians, and legal consultants) will be on hand to share their perspectives on the proper thought process for arriving at scientifically valid conclusions during mixture interpretation. The session will be driven primarily by questions solicited from DNA analysts ahead of time, from selected examples of complex mixtures, and from questions generated during the Special Session. The goals at the end of the session are for the audience to feel more confident in their approach to mixture interpretation based on the advice and experience provided by the panel and to be aware of the options available for statistical modeling of PCR-based DNA data. As a result, the forensic DNA community may grow or "transform" to keep in step with the complex types of mixtures that are becoming more prevalent in routine casework.

Mixture Interpretation, Probabilistic Genotyping, Statistics

B175 Optimization and Validation of Mitochondrial DNA (mtDNA) D-Loop Sequencing on the MiSeq®

Laura A. Wilson, BS, Penn State University, 107 Whitmore Lab, University Park, PA 16802; Sarah Copeland, BS, 300 Farmstead Lane, #8, State College, PA 16803; Gloria Dimick, MS, 2565 Park Center Boulevard, Ste 200, State College, PA 16801; Charity A. Holland, MPH, Mitotyping Technologies, 2565 Park Center Boulevard, Ste 200, State College, PA 16801; Robert Bever, Mitotyping Technologies, 2565 Park Center Boulevard, Ste 200, State College, PA 16801; and Mitchell M. Holland, PhD, Penn State University, 107 Whitmore Laboratory, University Park, PA 16802*

After attending this presentation, attendees will better understand how transcriptase-adapted Polymerase Chain Reaction (PCR) primers compare to the conventional PCR primers used today by forensic laboratories in the sequencing of the mitochondrial D-loop and the impact this comparison will have on the forensic science community.

This presentation will impact the forensic science community by aiding forensic laboratories in the adoption of a next generation sequencing approach when using the human mitochondrial D-loop protocol from the Illumina® MiSeq® instrument. Through heteroplasmy detection and reporting, sequencing the D-loop with a next generation approach on the MiSeq® will also increase the discrimination power of the testing method.

The mtDNA is present in high copy numbers, making it a powerful tool for analyzing forensic samples such as hair shafts and aged skeletal remains.[1,2] The non-coding region, or D-loop, is the target most often analyzed by forensic laboratories and contains two hypervariable regions known as HVR1 and HVR2; 16,024-16,365 and 73-340, respectively.[3] Sequencing the D-loop with a next generation approach on the MiSeq® will increase the discrimination power of the testing method via heteroplasmy detection and reporting. The D-loop protocol from Illumina® utilizes two sets of conventional, overlapping PCR primer pairs that span the hypervariable regions, with each primer possessing a transposase sequence added to the 5'-end that allows for library preparation prior to analysis on the MiSeq®.[4]

The first-round PCR amplification with Transposase Adapted (TA) primers was optimized and compared to the conventional primer pairs used today by forensic laboratories. The main difference between the two protocols (besides the modified primers) is the use of AmpliTaq® Gold® DNA polymerase versus Ex Taq™ Hot Start from TaKaRa, a polymerase with 3' to 5' exonuclease proofreading activity that utilizes an optimized Ex Taq™ buffer system.[5] The limitations of the PCR reaction parameters were also tested; for example, primer concentrations, magnesium concentration, and the amount of Ex Taq™ employed. The optimized amplification was used in validation studies performed by this group on the Illumina® D-loop protocol following the Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines. The studies included: (1) the evaluation of the robustness of the first-round PCR amplification when using the TA primers or the conventional primer pairs; (2) the sensitivity of library preparation by adding a range of DNA (amplicon) inputs; (3) mixture studies with various ratios of contributor DNA; (4) the evaluation of precision and accuracy through repeatability (same operator and detection instrument); and, (5) concordance experiments. These findings represent an important step toward the adoption of a next generation sequencing approach by forensic laboratories by using the D-loop protocol from Illumina® on the MiSeq® instrument.

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Next Generation Sequencing, Mitochondrial D-Loop, PCR

B176 Massively Parallel Sequencing (MPS) of Microhaplotypes for Forensics

Sharon C. Wootton, PhD, 180 Oyster Point Boulevard, South San Francisco, CA 94080; Kenneth Kidd, PhD, Yale University School of Medicine, Dept of Genetics, 333 Cedar Street, New Haven, CT 06520; William C. Speed, PhD, Yale University, 333 Cedar Street, New Haven, CT 06520; Joseph P. Chang, BS, Thermo Fisher Scientific, 180 Oyster Point Boulevard, South San Francisco, CA 94080; Sheri J. Olson, MS, Thermo Fisher Scientific, 409 Roosevelt Boulevard, Half Moon Bay, CA 94019; Reina Langit, MS, 850 Lincoln Centre Drive, Foster City, CA 94404; Chien-Wei Chang, PhD, Thermofisher Scientific, 180 Oyster Point Boulevard, South San Francisco, CA 94080; and Robert Lagace, BS, 850 Lincoln Centre Drive, Mail Stop #404-1, Foster City, CA 94404*

After attending this presentation, attendees will better understand the fundamentals of MPS and its application to forensics, particularly the use of microhaplotypes for determining biogeographic ancestry and for resolving mixture and kinship scenarios.

This presentation will impact the forensic science community by demonstrating microhaplotype multi-allelic genomic markers as a forensic solution to be used in conjunction with other Single Nucleotide Polymorphism (SNP) and Short Tandem Repeat (STR) markers in an MPS context. This presentation will show analysis from experiments designed to test the sensitivity of mixture detection, to define the ancestry prediction resolution, and to measure the accuracy of kinship analysis.

Recent studies demonstrating the use of MPS in forensics have shown a new methodology for interrogating a wide range of genomic markers beyond STRs, including mitochondrial DNA(mtDNA), messenger RNA (mRNA), and SNPs for identity, biogeographic ancestry, and phenotype.[1,2] The flexibility of a small amplicon, highly multiplexed, genomic assay enables the analysis of degraded or low template DNA samples. The additional resolution of sequence differences plus fragment length for an STR locus supplements the analysis of familial relationships and the deconvolution of mixtures.

When multiple SNPs reside within a single sequencing read (~<300bp), there may be multiple haplotypes represented as statistically phased SNP genotypes.[3] These multiallelic markers, like STRs, provide an excellent tool for kinship and mixture analysis. Additionally, some microhaplotypes may be used to derive biogeographic ancestry when there is large enough allele frequency variation between global populations.[3]

Forty-five microhaplotypes with high heterozygosity were selected using a set of criteria defined by Dr. Kenneth Kidd.[3] All of these have been run on a set of 54 populations by the Kidd laboratory. Primers were designed to amplify all markers in multiplex. DNA was extracted from a number of sample sources taken from individuals of different biogeographic ancestries. Mixtures were created at ratios of 1:1, 1:3, 1:7, 1:15, and 1:30. Samples were also selected to represent a number of kinship scenarios. Libraries for MPS were created by ligating barcoded sequencing adaptors. The barcoded libraries were sequenced on the Ion Torrent™ PGM™. Sequencing reads were aligned to target regions of the reference human genome; haplotypes were determined; and tertiary analysis was performed to type biogeographic ancestry, mixture ratios, minor and major contributors, and familial relationships. The multiplex was able to amplify and sequence all intended targets with <10% off-target amplicons. There was no ambiguity-calling haplotypes, and further analysis showed promise for resolving mixture components, ancestry, and kinship.

With the capacity to sequence many markers in parallel, MPS underscores the power of microhaplotypes as a forensic marker. Its use in multiplex with STRs and lineage, phenotype, and ancestry-informative SNPs could be investigated for a comprehensive forensic solution.

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Massively Parallel Sequencing, Microhaplotypes, Ancestry

B177 Optimization of a Next Generation Sequencing (NGS) Protocol for Processing High-Quality Mitochondrial DNA (mtDNA) Samples

Joseph D. Ring, MS, 115 Purple Heart Drive, Dover AFB, DE 19902; Michelle A. Peck, MFS*, Armed Forces DNA Identification Laboratory, 115 Purple Heart Drive, Dover AFB, DE 19902; Erin M. Gorden, MFS*, Armed Forces DNA Identification Lab, 115 Purple Heart Drive, Dover AFB, DE 19902; Charla Marshall, PhD, Armed Forces DNA Identification Laboratory, 115 Purple Heart Drive, Dover AFB, DE 19902; and Kimberly S. Andreaggi, MFS*, ARP/AFDIL, 115 Purple Heart Drive, Dover AFB, DE 19902*

After attending this presentation, attendees will better understand the differences that exist in sequencing mtDNA using two different library preparation kits that involve enzymatic fragmentation. Additionally, attendees will learn about an optimized mtDNA library preparation protocol for sequencing on the Illumina® MiSeq®.

This presentation will impact the forensic science community by introducing an effective method for library preparation and sequencing of high-quality mtDNA samples. The kit comparisons and optimizations made will help other forensic DNA laboratories with their transition to NGS processing.

The role of the Armed Forces Medical Examiner's/Armed Forces DNA Identification Laboratory (AFMES/AFDIL) is to aid in the identification of United States service members from current and past conflicts. Past accounting DNA testing support requires a substantial amount of mtDNA reference data production. NGS improves on the current Sanger-type sequencing methodology by allowing for higher throughput and automated analysis workflows. This high-throughput capability is especially useful for reference and population sample processing, which can be time consuming with current techniques. Two kits (Illumina's® Nextera® XT and KAPA HyperPlus by KAPA Biosystems) were evaluated that can be utilized for sample library preparation for sequencing on an Illumina® MiSeq®. Protocols for these kits were optimized for both the mtDNA Control Region (CR) and the entire mitochondrial Genome (mtGenome).

The CR and mtGenome of positive control DNA and buccal swab extracts were amplified, and varying amounts of enriched product were used for the library preparation to test the range of inputs typically observed in processing. In addition to using the manufacturer's recommended full-volume reactions, kit volume reagents were reduced by half. Other conditions tested include whether clean-up steps were needed before library preparation and the optimal fragmentation conditions to produce an ideal fragment-size distribution. Sample libraries were sequenced on a MiSeq® instrument. The sample libraries prepared at full reagent volume produced similar fragment concentrations as those prepared using half-volume reagent volumes. The degree to which the sample library fragmented was found to be a useful indicator of sequencing success. Sample libraries that were fully fragmented had, on average, approximately a 200% increase in the number of sequence reads compared to those libraries that had partial to no fragmentation.

In conclusion, a viable method has been optimized for preparing and sequencing both the CR and mtGenome of high-quality reference-type samples on the MiSeq®. Reagent volumes are able to be reduced with no loss of data quality and the process is easily amenable to automation. These optimizations lower cost and increase the efficiency of the process. Sequencing the mtGenome is prohibitive with Sanger-type sequencing because it is expensive and labor intensive. NGS and the additional optimizations to the library preparation allow mtGenome sequencing to be just as practical as CR sequencing. These procedures improve upon current methodology and will help bolster identification efforts at the AFDIL.

The opinions or assertions presented hereafter are the private views of the authors and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the United States Army Medical Research and Materiel Command or the Armed Forces Medical Examiner System.

Next Generation Sequencing, Mitochondrial DNA, Library Preparation

B178 Assessing the Impact of DNA Damage on the Interpretation of Low-Level Mitochondrial DNA (mtDNA) Heteroplasmy

Molly M. Rathbun, BS, Penn State University, 107 Whitmore Lab, University Park, PA 16802; Jennifer A. McElhoe, DPhil, Pennsylvania State University, 107 Whitmore Lab, University Park, PA 16802; and Mitchell M. Holland, PhD, Penn State University, 107 Whitmore Laboratory, University Park, PA 16802*

After attending this presentation, attendees will understand the importance of considering DNA damage effects on Next Generation Sequencing (NGS) sequence data. Attendees will also be more aware of how these effects will be important when developing reliable guidelines for interpreting and reporting mtDNA heteroplasmy or other types of low-level Single Nucleotide Polymorphisms (SNPs) in future forensic casework. This presentation will include a discussion on extract storage conditions, characteristics and identification of false low-level variant positions, and interpretation threshold recommendations.

This presentation will impact the forensic science community by elucidating characteristics of DNA damage in the mtDNA control region as it relates to the interpretation of heteroplasmy when using the NGS technology from Illumina®, the MiSeq®. With the introduction of NGS to forensic laboratories in the near future, the effect of DNA damage on any NGS sequence data should be characterized in order to prevent reporting of false positive sequence data. Given that low-level variants have the potential to make mtDNA more discriminating by offering the ability to better distinguish between unrelated individuals and maternal family members and by offering a more statistically significant likelihood ratio, it becomes inherently important to understand how damage affects these interpretations when made in modern forensic investigations and identifications. In addition, this information will contribute to understanding how damage may impact the analysis of nuclear DNA SNPs. There is confidence that this study of the impact of DNA damage on mtDNA heteroplasmy observations will help lead to recommendations of best practices for NGS forensic applications.

Forensic mtDNA analysis is a robust technique that is advantageous for challenging samples, but the identification through maternal haplotypes limits the discrimination potential compared to Short Tandem Repeat (STR) analysis. Heteroplasmic sequence variants can potentially provide distinction between maternal relatives and significantly increase likelihood ratios associated with matching mtDNA profiles; however, the nature of heteroplasmy as mixtures means that other sources of mixtures, such as DNA damage, must be eliminated as the cause of an apparent variant.[1] DNA damage is frequently encountered in forensic mtDNA analysis, so it is important to understand its effect on the interpretation of heteroplasmy. NGS of DNA now offers higher sensitivity than the Sanger method, allowing for detection of low-level heteroplasmy with a 1% minor variant.[2,3] Given that damaged sites can be observed with the Sanger method of sequencing, it was anticipated that damage will impact heteroplasmy interpretation using an NGS approach. Considering that mtDNA heteroplasmy may someday be more widely reported in forensic casework, a clear understanding of how damage-related anomalies affect NGS data will be important to the forensic community.

The goal of this study was to characterize the impact of DNA damage on the interpretation of mtDNA heteroplasmy, mainly in regard to low-level variants. The conditions that encourage damage mechanisms, such as deamination, which may arise from storage of DNA extracts or postmortem exposure to the element, were modeled.[4,5] Samples were run on the Illumina® MiSeq® following Nextera® XT library preparation. The sequencing data showed concordance with the Sanger method, providing consistent haplotypes across all conditions and maintaining the reliability of common DNA extract storage practices; however, it was discovered that when using an interpretation threshold of 1% for low-level heteroplasmy, as damage conditions become more intense, the number of apparent heteroplasmic positions increased. A decrease in the expected transition/transversion ratio was observed, suggesting that the sites of damage were random in relation to normal mutation mechanisms. Also, most of the false heteroplasmic positions showed a 1%-2% minor variant, with certain positions more likely to show false heteroplasmy. This may be useful for separating out and reporting true variants as interpretation criteria are developed.

Altogether, the results of this study of DNA damage advocate for careful consideration of long-term extract storage methods. NGS data produced from challenging evidence samples such as these will impact threshold considerations for reporting low-level heteroplasmy in future mtDNA casework. This assessment will also help contribute to the recommendations of best practices for forensic NGS-reporting guidelines as this technology is incorporated into crime laboratories.

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MiSeq®, MtDNA Heteroplasmy, DNA Damage

B179 Comparison of DNA Repair Methods for Improved Success With Next Generation Sequencing (NGS) of Compromised Skeletal Remains

Erin M. Gorden, MFS, Armed Forces DNA Identification Lab, 115 Purple Heart Drive, Dover AFB, DE 19902; Charla Marshall, PhD*, Armed Forces DNA Identification Laboratory, 115 Purple Heart Drive, Dover AFB, DE 19902; and Kimberly S. Andreaggi, MFS*, ARP/AFDIL, 115 Purple Heart Drive, Dover AFB, DE 19902*

After attending this presentation, attendees will be aware of the existence of DNA damage in common forensic samples in both Sanger sequencing and NGS data. This presentation will address ways to overcome this damage using DNA repair kits.

This presentation will impact the forensic science community by demonstrating how DNA damage from common forensic samples (such as bones and teeth) can impact analysis of both Sanger and, to a larger degree, NGS results. This presentation will also exhibit the ability of various kits to repair the damage.

The Armed Forces DNA Identification Laboratory (AFDIL) was established with the primary purpose of employing DNA techniques to assist in the identification of remains of United States service members. The identification of compromised skeletal remains by the AFDIL's past accounting section is achieved by using a combination of analysis types: mitochondrial DNA (mtDNA), autosomal Short Tandem Repeat (STR), and Y-chromosomal Short Tandem Repeat (Y-STR) testing; however, the condition of skeletal remains, including exposure to environmental insults and chemical treatment, can present a challenge in obtaining results due to DNA degradation and Polymerase Chain Reaction (PCR) inhibition. NGS can aid in the identification process by yielding results for such damaged and degraded samples in a more cost-effective and high-throughput manner than current methodologies. For these reasons, NGS methods are currently being optimized and validated for degraded DNA casework at the AFDIL.

Several skeletal elements that spanned a quality range typically encountered in past accounting cases as well as appropriate controls were enriched for the mtDNA control region using mini-primer sets (125bp-180bp). Following PCR enrichment, library preparation was performed for sequencing on the Illumina® MiSeq®. The NGS data for the positive controls were of good quality and generated the expected profiles; however, erroneous variants were detected at nearly every position across the targeted regions for the low-quality casework samples. These errors were observed at variant frequencies as high as 30% and consisted mostly of GàA and CàT mutations, which is indicative of DNA damage due to cytosine deamination. Sanger sequence data confirmed the presence of DNA damage, but at a level that did not impact variant calling due to the qualitative nature of this type of analysis. Furthermore, a decrease in Guanine-Cytosine (GC) content was observed in the sequences from the skeletal samples, providing additional evidence of DNA damage.

To address the DNA damage in casework samples, two methods of DNA repair were evaluated: the NEBNext® Formalin-Fixed Paraffin-Embedded (FFPE) DNA Repair Mix and Uracil-Specific Excision Reagent (USER™) Enzyme. In an initial study, improved sequence results were obtained from six casework samples that underwent repair treatment in comparison to the untreated extracts. Approximately 20% of sequences in repaired samples showed a shift in GC, indicating damage, compared to 70% in untreated samples. The number of mapped reads generated with NEBNext® FFPE repair-treated samples was closest to that of the untreated samples (two to three times higher than those treated with the USER™ enzyme), resulting in greater coverage across the targeted regions. The low number of reads obtained with the USER™ protocol is most likely the result of a required clean-up step in which DNA loss is expected. This step is not necessary in the FFPE protocol, making it a more attractive option for use with low-quantity samples. In most cases, unauthentic low-level variants observed in the untreated samples were eliminated in the repaired extracts. The results of this work suggest that DNA repair of compromised samples will not only be necessary for NGS processing but will also be beneficial for current Sanger sequencing of skeletal remains performed at the AFDIL. As the forensic community continues to look toward NGS for its increased sensitivity and low-level variant detection capability, DNA damage in low-quality specimens must first be addressed in order to generate reliable data.

The opinions or assertions presented herein are the private views of the authors and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the United States Army Medical Research and Materiel Command or the Armed Forces Medical Examiner System.

Next Generation Sequencing, DNA Damage, DNA Repair

B180 Sequence-Based Analysis of Stutter at Short Tandem Repeat (STR) Loci: Implementation and Utilization

Rachel Aponte, 9815 Bristol Square Lane, Apt 301, Bethesda, MD 20814; Katherine B. Gettings, PhD, NIST, 100 Bureau Drive, MS 8314, Gaithersburg, MD 20899; David L. Duewer, PhD, 100 Bureau Drive, Gaithersburg, MD 20899; Becky Hill, MS, 100 Bureau Drive, MS 8311, Gaithersburg, MD 20899; Michael D. Coble, PhD, 100 Bureau Drive, MS 8312, Gaithersburg, MD 20899-8312; and Peter M. Vallone, PhD, 100 Bureau Drive, Gaithersburg, MD 20899-8311*

After attending this presentation, attendees will understand the properties of Polymerase Chain Reaction (PCR) stutter artifacts observed in data obtained using Next Generation Sequencing (NGS) technologies and Capillary Electrophoresis (CE) methods.

This presentation will impact the forensic science community by laying a foundation for artifact interpretation guidelines, which will be required prior to implementation of NGS technologies. Observing how stutter artifacts characterized by NGS systems compare to those in traditional CE systems will help establish these guidelines. The characterization of stutter with NGS will aid in single-source data interpretation, mixture interpretation, and allow for sequence-based stutter thresholds to be set.

NGS technologies provide the potential for deeper analysis of forensic DNA samples compared to the currently employed length-based CE methods because NGS yields the full DNA sequence of each allele opposed to only the number of repeat units obtained by CE. Access to the full sequence can further individualize a DNA fingerprint by allowing specific base changes or changes within the repeat patterns of more complex loci to be observed. This is particularly useful when multiple alleles at a locus overlap with length-based genotyping but can be distinguished with NGS based on differences within the sequence motif.

NGS systems hold promise for advances in forensic DNA typing, but they will require a new understanding of artifacts and the corresponding interpretation guidelines in order to be beneficial for the forensic community. Stutter artifacts may behave differently with NGS data than what has been observed in CE data due to the differences in the system workflows. Characterization of stutter events between NGS and CE was obtained using the Promega® AutoSeq™ and PowerPlex® Fusion STR multiplex amplification kits, respectively. This study will be expanded to evaluate if stutter events are consistently observed across assays between the instrument platforms.

For the common tetra-nucleotide STR loci, stutter is commonly seen at the n-4 position in both CE and NGS data, but may be observed more frequently at the n+4 and n-8 positions in NGS data. It is possible that the higher frequency of stutter is due to a higher sensitivity of the instrumentation, but it is also possible that these go undetected due to the thresholds set for CE instruments. Lowering the threshold for CE data down to ten Relative Fluorescent Units (RFUs) allows for a better comparison with stutter from NGS systems, which currently have no established thresholds. Comparing stutter between the two systems will provide insight into how the differences of NGS workflows may affect artifacts, and thus, data interpretation. Analyzing each locus and the various types of stutter associated with each allele by sequence will allow for the establishment of allele- and sequence-specific stutter thresholds, which may further benefit current models used for mixture interpretation.

Access to the sequence of each allele also allows for general characterization of where stutter occurs within the repeat motif and whether it is primarily related to the Longest Uninterrupted Stretch (LUS) or to the total number of repeats within an allele.[1] Compound and complex loci — including D2S1338, D3S1358, D8S1179, D19S433, D21S11, FGA, and vWA — have an abundance of alleles with different motifs and would therefore benefit from sequence-based stutter thresholds. Observing the LUS of an allele opposed to observing alleles solely based on the total repeat number may provide a more accurate representation of stutter artifacts for each sequence. For example, results from data analysis of a 14 allele at locus D8S1179 demonstrates differences in stutter ratios when comparing simple and compound repeat motifs. The compound 14 allele with a LUS of 12 exhibited less stutter than the simple motif 14 allele. Therefore, targeting the LUS will allow for even more accurate stutter thresholds to be implemented.

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Next Generation Sequencing, Stutter Artifacts, STR Loci

B181 Light It Up: Fluorescent Biosensors for the Detection of Biological Fluids and Fingerprints

James Gooch, King's College London, 150 Stamford Street, Waterloo, London SE19NH, UNITED KINGDOM; Barbara Daniel, PhD, King's College London, 150 Stamford Street, Waterloo, London SE19NH, UNITED KINGDOM; Vincenzo Abbate, PhD, King's College London, 150 Stamford Street, Waterloo, London SE19NH, UNITED KINGDOM; and Nunzianda Frascione, PhD, King's College London, 150 Stamford Street, Waterloo, London SE19NH, UNITED KINGDOM*

After attending this presentation, attendees will better understand biosensing technology and its potential for application toward the simultaneous detection and identification of biological fluids as a replacement for traditional serological testing. Attendees will also be aware of how this approach can be extended to other forensically relevant targets, including fingerprints.

This presentation will impact the forensic science community by introducing new techniques that are able to offer the rapid, non-destructive, and highly specific multiplex analysis of biological fluids at crime scenes. It is hoped that this presentation will also encourage researchers to utilize biosensor technology for the detection of other forensic analytes in their relative fields of expertise.

The search for body fluids often forms a crucial element of many forensic investigations. Confirming fluid presence at a scene can not only support or refute the circumstantial claims of a victim, suspect, or witness, but may additionally provide a valuable source of DNA for further identification purposes; however, current biological fluid testing techniques are impaired by a number of well-characterized limitations: (1) they often give false positives (between fluids and other non-fluid substances); (2) they cannot be used simultaneously; (3) they are sample destructive; and, (4) they lack the ability to visually locate fluid depositions.[1] These disadvantages can negatively affect the outcome of a case through missed or misinterpreted evidence.[2,3]

Recent improvements in fluid assay specificity have utilized immunological testing strips for the detection of fluid-endogenous protein biomarkers; however, these testing processes do not allow for the retention of fluids following application, potentially sacrificing the opportunity for genetic profiling.[4,5] The direct detection of these proteomic markers without the removal and possible destruction of fluids may be achieved through "biosensing," in which biological interactions are transduced into observable signal outputs within the same molecular unit. High specificities make both antibody- and enzymatic-sensing elements ideal candidates for fluid analysis, while changes in fluorescence intensity upon target interaction may allow the visualization of *in situ* fluid depositions. Furthermore, the simultaneous detection of multiple fluid analytes may potentially be achieved by exploiting fluorophores of differing wavelengths in a single multiplex assay.

This study utilized two biosensing mechanisms toward the detection of biological evidence. Two fluorogenic peptide substratesreagents specific to Prostate Specific Antigen (PSA) and Kalikrein 8 (KLK8) were first utilized for the detection of human semen and sweat, respectively. Both substrates were able to successfully detect targets across a range of surfaces typical to forensic investigation with additional visualization via a direct spraying application. Substrates exhibited ideal increases in fluorescence intensity upon target interaction, even at 1:1,000 fluid dilutions, providing an opportunity for their use in contaminated deposits or those washed in removal attempts. Furthermore, promising results were displayed in the design and development of a customized displacement immunosensor specific to human semen, which was able to identify nM amounts of PSA within solution. Importantly, both sensing mechanisms explored were found to have no effect on DNA profiling processes after application to biological fluids, allowing the source of depositions to be identified without potential destruction of genetic material.

Displaying immediate and specific response to analyte presence, biosensors have the potential to prevent month-long visual evidence searches by localizing fluid depositions within a matter of seconds. Successful sensor employment is likely to lead to a significant reduction in the labor expense associated with current manual stain search and identification strategies.

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Body Fluids, Biosensors, Fingerprints

B182 Streamlining Sperm Cell Detection Via Proximity Ligation Real-Time Polymerase Chain Reaction (PLiRT-PCR) With Forensic DNA Analysis

Sarah Riman, PhD, 1009 New Hampshire Avenue, NW, Apt 1, Washington, DC 20037; and Daniele S. Podini, PhD*, Department of Forensic Science, 2100 Foxhall Road, NW, Washington, DC 20007*

After attending this presentation, attendees will gain valuable insight into the research efforts carried out to develop a PLiRT-PCR assay for the confirmatory detection of spermatozoa from sexual assault evidence without the use of a microscope.

This presentation will impact the forensic science community by demonstrating that PLiRT-PCR is a methodology compatible with current DNA Short Tandem Repeat (STR) analysis and is able to detect sperm-specific proteins by using only 2µL of a forensic sample.

Sperm identification plays a critical role in forensic investigation for understanding the circumstances surrounding a crime and determining whether or not a sexual act occurred, yet current methods for sperm detection vary widely in speed, sensitivity, and specificity and can sometimes lack the ability to confirm that the test results are conclusive.

Presently, laboratories routinely use an alternate light source as an enhancement tool followed by presumptive testing of semen using the Seminal Acid Phosphatase assay. The next step can be to test for Prostate Specific Antigen (PSA/p30) with commercial immunochromatography strips; however, the only undisputable confirmatory test for the presence of semen is the microscopic observation of spermatozoa. This process can be extremely time consuming and labor intensive, and failure to identify sperm cells by microscopic examination is not conclusive for their absence.

A potential solution to this issue is PLiRT-PCR, which is designed to detect and quantitate the expression of protein markers through an antibody-protein binding reaction followed by PCR. In fact, the assay combines the specificity of an immunological reaction with the sensitivity of quantitative PCR (qPCR). Sperm-specific protein SP10 (ACRV1) has been selected for this purpose. This target is only expressed in the male reproductive tract, specific to sperm cells, and localized inside the acrosome of the spermatozoa. This location protects SP10 from environmental damage until lysis, and thus allows for successful detection with aged forensic samples.

Probes are generated by conjugating polyclonal affinity purified SP10 antibodies to DNA oligos ending either in 3' or in 5'. When probes bind to their sperm target, the DNA strands attached to the antibodies come into close proximity and bind to a complementary connecting oligo added to the solution. These oligos are then ligated, forming a new amplifiable DNA strand that can then be detected by TaqMan® real-time PCR. The quantity of the amplified DNA corresponds to the amount of SP10, which is proportional to the amount of sperm cells in the sample. Results are determined based on a Cycle Threshold (C_T) value derived from three times the standard deviation from the "No Protein Control" (NPCs) C_T average.

This presentation discusses the identification, specificity, and the limit of detection of SP10 in: (1) liquid semen; (2) semen elution from cotton swabs; (3) pure body fluids; and, (4) mixed body fluid samples in a 96-well format. Most importantly, these experiments illustrate how PLiRT-PCR can be used to streamline the workflow of sperm confirmation with the generation of a DNA profile of the perpetrator(s) involved in the sexual assault. The assay utilizes a small fraction of the total reaction. Thus, the remaining can then be used for downstream DNA extraction, quantitation, and STR amplification.

In conclusion, this method is robust, quantitative, and more sensitive than the currently used protein-based detection techniques; samples can be processed in parallel on a 96-well plate for high-throughput analysis with minimal sample consumption. In addition, it can overcome the drawbacks associated with the microscopic observation of spermatozoa and can easily be integrated into forensic laboratories as it only requires a thermocycler and a real-time PCR system.

Spermatozoa, Sexual Assault, Proximity Ligation

B183 Developmental Validation of MicroRNAs (miRNAs) for Body Fluid Identification

Carolyn Lewis, BS, Virginia Commonwealth University, 1015 Floyd Avenue, Rm 2004, Box 843079, Richmond, VA 23284; Jamie Gentry, BS, Virginia Commonwealth University, 1015 Floyd Avenue, Richmond, VA 23284; Chelsea F. Calloway, BS, 1005 Grove Avenue, #102, Richmond, VA 23220; Nerissa Peace, BS, 3270 John Robinson Lane, #12, Dumfries, VA 22026; Ariana Albornoz, MS, 3660 E Bay Drive, Apt 1316, Largo, FL 33771; Samantha R. Fleming, MS, 10306 Gunston Road, Lorton, VA 22079; Christina Hayes Nash, MS, 17901 Milroy Drive, Dumfries, VA 22026; Zendra E. Zehner, PhD, VCU School Med/Massey Cancer Center, Dept of Biochemi & Molecular Biology, 1101 E Marshall Street, PO Box 980037, Richmond, VA 23298-0037; and Sarah J. Seashols Williams, PhD, Virginia Commonwealth University, Dept of Forensic Science, PO Box 843079, Richmond, VA 23284-3079*

After attending this presentation, attendees will understand how miRNAs function and why they can be of significant value for body fluid identification in forensic casework. Attendees will be apprised of the markers that can distinguish different body fluids and understand why miRNAs may be a better molecular-based method for the identification of body fluids rather than the use of current serological tests, which are based on enzymatic activity and are often prone to false positives. Although molecular-based methods of identification have previously been introduced into the forensic science community, the research to discover body fluid-specific miRNAs was based on other methods and the data was often conflicting. Attendees will understand the concept of miRNAs as molecular markers for body fluids and be encouraged to promote the necessary research for implementation of miRNAs in forensic casework.

This presentation will impact the forensic science community by illustrating how forensic research on miRNAs continues to build evidence for their utility as forensic molecular markers.

MicroRNAs are small non-coding RNAs that are 18 to 22 nucleotides in length and have previously been identified as potential markers for the identification of forensically relevant body fluids. There is an increased interest in the use of miRNAs because their short length, cellular function, and resistance to degradation allow for easy detection in highly degraded samples, as is often the case in forensic casework samples.

High-Throughput Sequencing (HTS) of three to four donations each of feces, urine, peripheral blood, menstrual blood, vaginal fluid, semen, saliva, and sweat was performed. The data analysis identified several candidate miRNAs with potential body fluid specificity. Initial Real-Time quantitative Polymerase Chain Reaction (RT-qPCR) evaluations revealed that while no evaluated miRNA was absolutely body fluid specific, miRNAs were identified in most body fluids with significantly different relative expression levels, which allows for body fluid identification using a panel of miRNAs. Each of the candidate miRNAs was evaluated thoroughly using classic developmental validation methods including species specificity, limit of detection, abundance within the population, and abundance within an individual over a certain period of time. The miRNAs let-7g and let-7i were identified and validated as candidate miRNAs for internal control purposes as they were expressed with consistent levels in each body fluid from all tested donors. A standard curve using a synthetic miRNA of known quantity was developed to gain a more accurate quantitation method. Based on these data, miRNAs, or the combination of certain miRNAs, can be valuable forensic molecular markers for body fluid identification, especially in degraded or low copy-number samples.

MicroRNA, Body Fluid Identification, Validation

B184 Recent Progress in the Development of a Surface-Enhanced Raman Spectroscopy (SERS) Platform for Rapid Identification of Trace Amounts of Human Body Fluids

Jennifer Fore, PhD, Boston University, Photonics Center-Dept of Chemistry, 8 St. Mary's Street, Boston, MA 02118; Ranjith Premasiri, PhD, Boston University, Dept of Chemistry, 590 Commonwealth Avenue, Boston, MA 02215; Kathryn A. Zegarelli, BS, 55 Deerfield Street, #2, Boston, MA 02215; Brandon Scott, PhD, Boston University, 8 St. Mary's Street, Boston, MA 02215; Jessica Irvine, BS, Boston University School of Medicine, 72 E Concord Street, Boston, MA 02118; Amy N. Brodeur, MFS, Boston University School of Medicine, Biomedical Forensic Sciences, 72 E Concord Street, R806, Boston, MA 02118; and Lawrence Ziegler, PhD, Boston University, Dept of Chemistry, 590 Commonwealth Avenue, Boston, MA 02215*

After attending this presentation, attendees will gain an appreciation for the developing advances in a SERS-based platform that may result in a new capability for forensics investigators at crime scenes to detect and identify trace amounts of human body fluids with a rapid, easy-to-use single instrument.

This presentation will impact the forensic science community by providing quantitative results of the use of a SERS-based methodology for the identification of dried blood, semen, saliva, vaginal fluid, menstrual blood, and urine from a single portable platform. No such capabilities currently exist in the forensic science community.

The identification of trace amounts of human body fluids, such as blood, semen, vaginal fluid, saliva, and urine, is often central to understanding events at a crime scene. Currently, a variety of tests for detection and confirmatory identification for each of the body fluid types may be carried out. For example, Kastle-Meyer, Takayama, or antibody immunochromatographic strip tests for blood, Prostate Specific Antigen (PSA) detection for semen, Lugol's iodine reaction or messenger RNA (mRNA) -based hormone detection for vaginal fluid, and alpha amylase detection of saliva; however, these individual tests have limitations in terms of sensitivity, specificity, and on-site portability in addition to speed. A portable SERS-based methodology offers the possibility of a single-platform instrument for confirmatory identification at crime scenes with higher sensitivity and specificity and is faster than techniques currently commercially available to crime scene forensic investigators. Progress in the development of a portable, universal body fluid-detection platform is described here.

SERS spectra of dried blood, semen, vaginal fluid, saliva, and urine from multiple donors (360 spectra) were collected by a simple sample protocol which uses 1nL-100nL of body fluid and exhibits reproducible SERS spectra on Au nanostructured substrates.[1] A Partial Least Squares Discriminant Analysis (PLSDA) -based classification procedure results in 98.0% sensitivity and 99.5% specificity. The novel aspect of this classification procedure that permits this robust performance is that the inputs to the PLSDA are barcode representations based on the second derivative of the SERS spectra.[2] In a further test of methodology, 60 SERS spectra of semen from multiple donors that were not used to build the model were used to challenge this identification procedure. The PLSDA correctly identifies 58/60 SERS spectra, and the resulting set of 360 spectra shows (cross-validation) 96.7% sensitivity and 99.2% specificity.

When menstrual blood is added to this developing SERS library, it is identified as a separate body fluid and the resulting six body fluid PLSDA-classification model results in 96.1% sensitivity and 99.2% specificity. Acquisition of each spectrum requires about 10sec of data collection time and illumination by ~1mW of 785nm laser power, thus enabling the rapid and readily portable nature of this technique.

By expanding the size of the data base, spectral variation due to donor variability was found to be limited sufficiently to allow robust identification of human body fluid types. Several key components of each body fluid have been identified. Molecular vibrational signatures of uric acid, hypoxanthine, protein, and hemoglobin are seen in dried blood SERS spectra; protein, hypoxanthine, and adenine are seen in vaginal fluid spectra; protein, hypoxanthine, and xanthine are seen in semen; and phenylalanine and protein are seen in saliva. These studies intend to show that the SERS spectra of dried blood, vaginal fluid, and semen on various materials such as fabrics, carpet, or glass will have minimal variation and allow identification via the barcode-based PLSDA procedure. Mixture resolution of interest to sexual assault cases (i.e., semen and vaginal fluid, menstrual blood and semen, saliva and vaginal fluid) will be demonstrated.

The quantitative results presented will demonstrate that these most recent advances in the development of SERS for confirmatory identification of trace amounts of body fluids at crime scenes has the potential to be a rapid, sensitive, easy-to-use, portable tool for forensic investigators in the near future.

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SERS, Body Fluids, Identification

B185 Optimum Case Detection Limit of the Forensic Luminol Test for Bloodstains

Stephen L. Morgan, PhD, University of South Carolina, Dept of Chemistry & Biochemistry, 631 Sumter Street, Columbia, SC 29208; Brianna M. Cassidy, BS, University of South Carolina, Dept Chemistry & Biochemistry, 631 Sumter Street, Columbia, SC 29208; Zhenyu Lu, BS, University of South Carolina, Dept of Chemistry & Biochemistry, 631 Sumter Street, Columbia, SC 29208; Jennifer P. Martin, BS, University of South Carolina, Dept of Chemistry and Biochemistry, 631 Sumter Street, Columbia, SC 29208; Shawna K. Tazik, BS, University of South Carolina, Dept of Chemistry and Biochemistry, 631 Sumter Street, Columbia, SC 29208; Katherine A. Witherspoon, BS, University of South Carolina, Dept of Chemistry and Biochemistry, 631 Sumter Street, Columbia, SC 29208; Katherine E. Kilgore, 704 Sailclub Road, Hartsville, SC 29550; Stephanie A. DeJong, BS, University of South Carolina, Dept Chemistry & Biochemistry, 631 Sumter Street, Columbia, SC 29208; Raymond G. Belliveau, BS, University of South Carolina, Dept Chemistry & Biochemistry, 631 Sumter Street, Columbia, SC 29208; and Michael L. Myrick, PhD, University of South Carolina, Dept of Chemistry & Biochemistry, 631 Sumter Street, Columbia, SC 29208*

After attending this presentation, attendees and especially crime scene investigators and DNA analysts will have a realistic description, based on systematic experimentation under controlled conditions, of the chemical behavior of luminol during reaction with low levels of blood.

This presentation will impact the forensic science community by providing information concerning the optimum-case Limit Of Detection (LOD) for bloodstains, which could impact interpretation of luminol tests conducted at crime scenes.

Luminol formulations are used by criminologists as a presumptive test for detecting latent bloodstains. The luminol test has been used by forensic investigators to aid in the discovery of bloodstains and to visualize blood spatter for more than 60 years. Many studies have been carried out which explore the luminol Limit Of Detection (LOD) for bloodstains; however, the luminol LOD is still elusive, having detection limits reported which range from100x to 5,000,000x dilute bloodstains. This range in reported luminol LODs stems from lack of experimental control: luminol applied to bloodstains is generally not accurately measured, the blood-luminol response is usually detected qualitatively using human observation, and bloodstain samples were produced without regard to the effect blood dilution has on spreadability. These factors do not allow for accurate determinations of LODs or effective comparisons of blood detection agents. Furthermore, published studies have not quantitatively defined the relationship between important variables involved in the blood-luminol reaction (such as bloodstain dilution, amount of luminol applied, age of luminol solution, etc.) and chemiluminescent response. Knowledge of how the blood-luminol response is affected will increase the utility of luminol, allowing investigators to make educated decisions both in luminol application technique and in luminol response analysis.

An experimental method was designed with heightened variable control, which renders an optimum-case luminol LOD and reveals a linear relationship between bloodstain dilution and chemiluminescent response. Blood solutions ranging from 10,000x to 40,000x were deposited on cotton T-shirt swatches using a patented stain barrier technique. The luminol formulation was applied to bloodstains in complete darkness and the response was measured using a digital Charge-Coupled Device (CCD) camera. Pixel intensity information was extracted from the resulting raw images and a linear relationship was discovered between bloodstain dilution and chemiluminescent response. An optimum-case LOD of approximately 100,000x was calculated.

Luminol, Limit of Detection, Bloodstains

B186 Time-Dependent Loss of Messenger RNA (mRNA) Transcripts From Forensic Samples Analyzed Using Next Generation Sequencing

Katelyn D. Weinbrecht, MS, 1923 S Jackson Avenue, Apt 38K, Tulsa, OK 74107; and Robert W. Allen, PhD, Oklahoma State University, Center for Health Sciences, 1111 W 17th Street, Tulsa, OK 74107-1898*

After attending this presentation, attendees will understand the relationship between mRNA degradation and age outside the body for body fluid stains and how this information may allow for estimating how long a biological sample has been at a crime scene.

This presentation will impact the forensic science community by providing tools with which to estimate how long evidence has been at a crime scene and by possibly improving interval-since-death estimates.

Forensic biology generally has a focus on DNA and the identification of the donor of a DNA sample recovered at a crime scene. Recently, RNA analysis has also demonstrated potential as a worthwhile analyte in the forensic biology laboratory. Applications for RNA analysis include the use of RNA to the identify the tissue source(s) of an evidentiary sample, perhaps assisting in determining the age of a biological sample and in a determining the cause of death through an analysis of expressed genes and how defects in gene expression may have contributed to the death (i.e., a molecular autopsy). Although recent research has indicated many possible forensic applications of RNA analysis, many questions remain concerning the behavior of RNA in degraded and limited samples. Specifically, the need remains for a thorough understanding of the differing patterns and rates of RNA degradation in postmortem and deposited samples.

The purpose of this research was to evaluate mRNA degradation in forensically relevant biological sample types (blood, saliva, semen, and vaginal fluid) utilizing next generation sequencing of fresh and aged samples. By incorporating a panel of synthetic well-characterized RNA sequences of known molar concentration with the initial mRNA preparation, it was possible to quantitatively compare samples stored for various lengths of time up to one year and measure the concentration of different transcripts over time. The mRNA transcripts from tissue-specific markers and those present in all tissue samples were examined. A significant number of tissue-specific transcripts were identified. Specifically, there were 1,449 blood-specific transcripts, 124 saliva-specific transcripts, 211 vaginal fluid-specific transcripts, and 1,712 semen-specific transcripts detected. In addition to the tissue-specific transcripts, there were 1,875 transcripts common to all of the sample types. Both the tissue-specific transcripts and the common transcripts provide an adequate population for the selection of transcripts that disappear over time in a predictable way. Transcripts of both types (tissue-specific and common) decreased in abundance over the one year of storage. Tissue-specific transcripts exhibited varying degradation rates, with times ranging from a rapid disappearance within 30 days of storage at room temperature to remaining stable over the course of one year at room temperature.

The mRNA degradation profiles obtained from this study can be used as a guide to gene expression patterns in different body fluid samples and to basal mRNA degradation rates in samples stored under non-hostile conditions (i.e., room temperature in low light). This guide can be compared to sample storage under conditions that may promote accelerated degradation. Once acceptable candidate genes with predictable degradation rates have been identified and characterized, real-time PCR assays can be developed and implemented more easily in the forensic biology laboratory for routine analysis of casework samples.

mRNA Sequencing, mRNA Degradation, Sample Age

B187 An Evaluation of the Differential Stability of Nucleic Acids in Biological Fluids Compromised by Environmental Exposure

Tiffany R. Layne, BS, 3906 Grovewood Road, Hopewell, VA 23860; Zendra E. Zehner, PhD, VCU School Med/Massey Cancer Center, Dept of Biochemi & Molecular Biology, 1101 E Marshall Street, PO Box 980037, Richmond, VA 23298-0037; and Sarah J. Seashols Williams, PhD, Virginia Commonwealth University, Dept of Forensic Science, PO Box 843079, Richmond, VA 23284-3079*

After attending this presentation, attendees will better understand how much of an effect the environment has on evidence and biological material through a discussion of relative degradation rates of DNA, messenger RNA (mRNA), and microRNAs (miRNAs). This presentation will also increase awareness of RNA use in the forensic community and promote research in novel body fluid identification methods.

This presentation will impact the forensic science community by clarifying relative stabilities between RNAs and DNA within the same sample and the stability of RNA for body fluid identification purposes.

The visualization, presumptive, and confirmatory tests used for body fluid identifications in forensic casework have remained static for many years. This is problematic because many of these tests are known to yield false positive results with other biological fluids, foods, or chemicals. Recent work in the forensic science field has explored RNAs for a molecular-based, forensic body fluid identification method. Many researchers have assessed mRNA to identify body fluids. While these research studies provide good evidence for mRNA as being useful in body fluid identification, some research has provided unsupportive evidence and thus mRNA analysis has been slow to catch on for casework. The miRNAs are small RNAs that have the ability to suppress translation of mRNA into proteins and are shorter in length than mRNAs. Because of these advantages and the remarkable stability observed by the species, they are also being considered as markers for body fluid identification. This study evaluated the relative stability of DNA, RNA, and miRNAs in the same samples under conditions mimicking an outdoor crime scene.

Samples of blood, urine, semen, and saliva were placed in an environmental chamber for defined periods of time corresponding from 24 hours to 14 days during a Virginia summer. The environmental chamber manages the irradiance, air temperature, and humidity for a more controlled assessment of sample degradation. DNA and RNA were isolated from each stain, and quantitative Polymerase Chain Reaction (qPCR) for DNA and Real-Time (RT) qPCR for RNA and miRNAs was performed. Expression levels were calculated relative to the positive untreated control samples. DNA stability was evaluated using both standard TPOX and "mini" length primers for the Short Tandem Repeat (STR locus) TPOX. Both mRNA and miRNA expression were evaluated using GAPDH and/or ACTB, and Let-7g, respectively.

The miRNA expression was not significantly impacted by treatment in the environmental chamber, unlike the impacted levels of DNA and mRNA. When measuring miRNA stability, Let-7g levels were not significantly different from the untreated control levels for all four body fluid samples. The DNA and mRNA data showed how environmental effects can greatly degrade biological material after exposure. The data from this project drives home the environmental effects on biological material, clarifies differential stability of the nucleic acids, and, consequently, can provide the practitioner with options for analysis workflows in compromised samples.

RNA, Degradation, Body Fluid Identification

B188 Obtaining Significant Powers of Individual Discrimination From Hair Shaft Proteins

Glendon Parker, PhD, Protein-Based Identification Technology, 4421 Ashwood CMN, Fremont, CA 94538; Deon Anex, PhD, Lawrence Livermore National Laboratory, 7000 East Avenue, Livermore, CA 94550; Katelyn Mason, PhD, Lawrence Livermore National Laboratory, 7000 East Avenue, Livermore, CA 94550; and Bradley Hart, PhD, Lawrence Livermore National Laboratory, 7000 East Avenue, Livermore, CA 94550*

After attending this presentation, attendees will better understand the potential role of protein in obtaining quantifiable statistically based powers of discrimination from biological samples.

This presentation will impact the forensic science community by explaining how the past few decades have seen the impact of nuclear and mitochondrial DNA typing on forensic science. This presentation introduces a third identifying method based on protein typing. Genetically variant peptides from hair shafts can be used to infer the status of **Single Nucleotide Polymorphism** (SNP) loci in a subject's genome.

If DNA is not present in biological samples collected as evidence, the options for the forensic investigator are limited. Protein is considerably more stable and abundant than DNA by many orders of magnitude. Genetic variation found in protein encoding genes, in the form of non-synonymous SNPs (nsSNPs), results in changes in protein amino acid sequence. Identifying and detecting these single amino acid polymorphisms in proteins therefore allows genetic content of a subject's DNA to be inferred, allowing peptides to be a surrogate for absent or unusable DNA.

To demonstrate this approach, a thorough examination of the protein population in hair shafts was conducted. Hair shafts are a poor source of nuclear DNA, yet are present in many forensic and bioarchaeological contexts. Methods were developed to identify and maximally detect peptides in hair shafts that retain genetic information. First, hair is milled and extracted with high levels of reductant, urea, and mass spectrometry-compatible detergent. The proteins are then alkylated and digested with trypsin to produce a highly complex mixture of peptides. These peptides are then resolved by high-pressure liquid chromatography and analyzed by tandem mass spectrometry. The resulting mass spectra are then matched to peptide sequences and a subset of peptides containing single amino acid polymorphisms are identified.

Genetically Variant Peptides (GVPs) have been characterized that correspond to 43 genetic nsSNP loci. In applying the product rule, dependency between loci within the gene boundary and full independence beyond it were assumed for now. When the product rule is applied, these GVP profiles result in powers of discrimination up to 1 in 5.4 million in the European population. The efficacy of each GVP in inferring the genetic status of corresponding genomic SNPs can be directly measured when compared to direct Sanger sequencing of subjects' DNA. Out of 608 inferences made, only 12 were false positive assignments, a greater than 98% true positive rate. Importantly, false positives clustered around a limited number of less-predictive GVPs. When poorly performing GVPs are eliminated from the analysis, the false positive rate decreases to less than 1%. When using SNP allelic frequencies from the African population, the GVP profiles were considerably less common by a factor of up to 29,000. The resulting likelihood scores, including those from European and African subjects, are a source of biogeographic information that range more than seven orders of magnitude across the samples tested. This indicates that peptides have the potential to provide information about the genetic background of the hair donor. These numbers have been obtained by applying the equivalent of less than 2mm of hair shaft to mass spectrometric analysis.

Application of this methodology to forensic fieldwork samples requires two milestones to be achieved: information needs to be obtained from a single hair, and dependence patterns of hair shaft proteins need to be fully elucidated. Both of these projects are under active investigation. Expansion of these methodologies beyond hair proteins to include alternative tissue types such as teeth, bone, and skin cells is critical to expanding the scope and application space for this novel approach to human forensics.

Protein Typing, Hair, Proteomics

B189 Using DNA Barcoding to Assess DNA Viability in Plant and Insect Fragments Isolated From Forensic Soil Samples

Kelly A. Meiklejohn, PhD, ORISE/FBI Laboratory, 2501 Investigation Parkway, Quantico, VA 22204; Megan L. Jackson, BS, 1 Joplin Court, Stafford, VA 22554; Jack Hietpas, PhD, FBI-ORISE, 2501 Investigation Parkway, Quantico, VA 22135; Libby A. Stern, PhD, FBI Laboratory - CFSRU, 2501 Investigation Parkway, Quantico, VA 22135; and James M. Robertson, PhD, CFSRU, FBI Laboratory, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, attendees will understand the fundamentals of DNA barcoding and whether the DNA of biological fragments collected in forensic soil samples is viable toward molecular analysis.

This presentation will impact the forensic science community by providing an assessment as to whether the individual biological fragments commonly present in soil samples could provide additional information for provenance and thus be implemented in casework.

Every year, debris samples are submitted to forensic laboratories as evidence, but the insect and plant materials commonly found in such samples are rarely analyzed. This is despite the fact that individual insect and plant materials could add to the characterization of the sample that is provided by inorganic analysis.[1] In addition, biological material could provide information for soil geo-attribution, since insects and plants inhabit specific ecosystems. Seasonal distribution of plant and insect material will provide additional information for the sample. One reason such biological material is not analyzed is because microscopic identification is not straightforward, especially for examiners who not appropriately trained. Different individual specimens may present similar morphologies that are impossible to distinguish and there may be fragments instead of the whole insect or plant as well as specimens at different developmental stages. In these circumstances, using DNA for identification is an attractive alternative approach as DNA is present in all biological tissues.

Species-level identification is possible by using sequence data. DNA barcoding, which utilizes a standardized short sequence of DNA, typically 400–800 base pairs in length, is now a commonly accepted forensic approach for molecular identification.[2,3] The barcode sequence of the DNA from the item of interest is compared with sequences in public databases for classification of the sample. The mitochondrial Cytochrome Oxidase subunit 1 (CO1) gene has been adopted as the standard barcoding region for insect identification as it has a fast mutation rate and is found in high copies within tissues.[4] Two plastid regions (an organelle found exclusively in the cells of plants and algae), rbcL and matK, have been adopted as the core barcode regions for land plants as CO1 evolves too slowly to facilitate species-level discrimination of plant species.[4]

Using surface soil samples collected from various locations in Virginia with varied geology and ecohabitats to represent soil evidence associated with shoes and digging tools, the following will be discussed: (1) what types of plant and insect fragments are commonly recovered with surface soil samples; (2) whether such fragments contain viable DNA; and, (3) whether the appropriate DNA barcode regions could be amplified and sequenced using traditional Sanger methods. Ten insect and ten plant fragments were collected from each site (n=200). Various commercial kits for DNA extraction from the insect and plant material were compared. The results of these kits as well as the use of degenerate primers for COI, rbcL, and matK will be presented along with the success rate of the published barcoding protocols. Issues encountered when searching the data against two public databases, the National Center for Biotechnology Information (NCBI) and the Barcode of Life Database (BOLD) will be described in order to assess congruence and taxonomic identification.

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Forensic Soil Samples, Species Identification, DNA Barcoding

B190 Evaluation of a 13-Loci Short Tandem Repeat (STR) Multiplex System for *Cannabis Sativa* Genetic Identification

Rachel M. Houston, BS, 3505 Snidow Drive, Plano, TX 75025; Sheree R. Hughes-Stamm, PhD, Sam Houston State University, Dept of Forensic Science, Huntsville, TX 77341; and David A. Gangitano, PhD, Sam Houston State University, 13906 Paradise Valley Drive, Houston, TX 77069*

After attending this presentation, attendees will understand the basic principles behind using an STR multiplex method for individualizing marijuana samples.

This presentation will impact the forensic science community by providing an STR panel that could not only assist law enforcement agencies in verifying legal marijuana products but also aid in the linkage of illegal cases. This method could also serve as an additional tool to previously established marijuana profiling programs used in federal agencies such as the United States Customs and Border Protection (CBP) and the Drug Enforcement Administration (DEA).

Marijuana (*Cannabis sativa*) is the most commonly used illicit substance in the United States. Although the federal government considers marijuana a Schedule I substance, it has become legalized for recreational use in four states (Colorado, Washington, Oregon, and Alaska). As a result of legalization, law enforcement faces a unique challenge in tracking and preventing the flow of legal marijuana to states where it is illegal. Moreover, there is significant traffic of illegal marijuana from Mexico. When identifying marijuana for legal purposes, the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) recommendations require the confirmation of THC via Gas Chromatography/Mass Spectroscopy (GC/MS), the microscopic confirmation of the presence of cystolithic hairs, and a positive Duquenois-Levine color test.[1] These tests are sufficient for prosecuting an individual for possession of marijuana but do not provide any meaningful intelligence as to the origin or individualization of the plant; however, there are many methods that can be used to individualize and determine the origin of a marijuana sample. These methods include, but are not limited to, palynology, chemical profiling, Isotope Ratio Mass Spectrometry (IRMS), and DNA analysis.[2-6] DNA has been shown to provide higher resolution for the individualization of marijuana plants as compared to the other techniques.[7]

The development of a validated molecular method such as STRs could aid in the individualization of cannabis samples as well as serve as an intelligence tool to link multiple cases (e.g., illegal traffic at the United States-Mexico border). For this purpose, a modified STR multiplex method was optimized and evaluated according to the International Society for Forensic Genetics (ISFG) and the Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines.[8] A new quantitative Polymerase Chain Reaction (qPCR) method was developed to accurately quantitate the amount of cannabis DNA extracted. A sequenced allelic ladder was also designed to accurately genotype 199 *C. sativa* samples from 11 seizures provided by a federal agency.

Distinguishable DNA profiles were generated from 127 samples that yielded full STR profiles, and four duplicate genotypes within seizures were found. From the analysis of STR profiles and the lack of clonal material, it can be concluded that the analyzed cannabis samples, most likely from Mexico, were propagated from seeds. The combined power of discrimination of this multi-locus system is 1 in 70 million with a sensitivity of 0.25ng of template DNA. The 13-STR panel was found to be species-specific for *C. sativa*; however, non-specific peaks were generated for *Humulus lupulus* (Hops). Phylogenetic analysis and case-to-case pairwise comparison of 11 cases using F_{ST} as genetic distance revealed the genetic association of four groups of cases. Moreover, due to their genetic similarity (common origin), a subset of samples (*N*=97) was found to form a homogeneous population in Hardy-Weinberg and linkage equilibrium. Using this homogenous population, a reference cannabis population database with associated allele frequencies for forensic purposes was developed.

In conclusion, the results of this research demonstrate the applicability of this 13-loci STR system not only in associating cannabis cases for intelligence purposes, but also in potentially detecting the presence of plant material generated via clonal propagation.

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Forensic Botany, *Cannabis Sativa*, Short Tandem Repeats

B191 Development of a High Resolution Real-Time Polymerase Chain Reaction (PCR) Melt Assay for Identifying "Legal High" Plant Material

Alicia Quinn, BS, 120 Stoney Meadow Lane, Madison Township, PA 18444; and Kelly M. Elkins, PhD, Towson University, Chem Dept & Forensic Science Program, 8000 York Road, Towson, MD 21252*

After attending this presentation, attendees will better understand plants that endogenously produce chemicals used as "legal highs," how genetic targets can be probed using PCR, and how "legal highs" can be identified using high-resolution melt assays.

This presentation will impact the forensic science community by providing results of the development of a multiplex to probe several "legal high" plant species as well as specificity, reproducibility, selectivity, and sensitivity data for the singleplex assays. This study presents assays that could help forensic laboratories identify trace quantities of plant material in comingled samples.

Mixture analysis has been a cornerstone of human DNA typing methods for several years. Forensic laboratories focus a significant portion of their caseload on detecting and identifying controlled substances and new drugs of abuse. The interpretation of trace material is complicated by the fact that the quantity of recoverable material can fall below the detection limits of instrumental methods. While microscopy is used to identify botanical material, this method has limitations when only trace material is present. Similarly, Gas Chromatography/Mass Spectrometry (GC/MS) is used to identify the active drug compounds contained in the plant material when sufficient material is present for detection and identification. As PCR amplifies the extracted genetic material, it has the advantage of detecting trace or low-template DNA. High resolution PCR melt assays have been developed to detect and identify four plant species: *Cannabis sativa* (marijuana), *Papaver somniferum* (poppy), *Ipomoea purpurea* (morning glory), and *Datura stramonium* (jimson weed). Marijuana and poppy are the two most prevalent internationally abused herbal highs; jimson weed and morning glory can be found in gardens and along roadsides. Reports have indicated that users consume the seeds or plant material whole or via teas or smoking.

To differentiate the plants, PCR primers specific for each plant were designed using public genome data from the National Center for Biotechnology Information (NCBI) and evaluated uniqueness using the Basic Local Alignment Search Tool (BLAST). Each set of PCR primers was designed to produce an amplicon from the plant of interest with a specific melt temperature that differs from amplicons produced with primers for the other plants. DNA was extracted from plant and seed material using the QIAGEN® DNeasy® Plant Mini Kit and primers were obtained from IDT. Using the Bio-Rad iTaq™ Universal SYBR® Green Supermix, the primers were tested for specificity, selectivity, sensitivity, and reproducibility in singleplex reaction assays. Agarose gel electrophoresis was used to confirm production of PCR amplicons of the designed sizes. Progress in multiplexing primer sets to simultaneously detect one or more of the species using an LCGreen mastermix will also be presented. The PCR melt assay is an inexpensive, quick, and specific method to detect and identify genetic material derived from "legal highs." It is expected that the assay could be employed by forensic laboratories to detect and identify "legal highs" in the current international drug market.

DNA, PCR, High-Resolution Melt

B192 Epigenetic-Aging-Signature — The Future?

Athina Vidaki, PhD, King's College London, 150 Stamford Street, Franklin Wilkins Bldg, London SE1 9NH, UNITED KINGDOM; Anastasia Aliferi, King's College London, 150 Stamford Street, Franklin Wilkins Bldg, London SE1 9NH, UNITED KINGDOM; David Ballard, PhD, King's College London, 150 Stamford Street, London SE1 9NH, UNITED KINGDOM; Leon Barron, PhD, King's College London, 150 Stamford Street, Franklin Wilkins Bldg, London SE1 9NH, UNITED KINGDOM; and Denise Syndercombe Court, PhD, King's College London, 150 Stamford Street, London SE1 9NH, UNITED KINGDOM*

After attending this presentation, attendees will better understand the potential of applying epigenetic markers in forensic age prediction.

This presentation will impact the forensic science community by proposing a quantitative Next Generation Sequencing (NGS) approach in estimating chronological age and by demonstrating this approach's accuracy, reproducibility, and applicability in a set of whole blood samples and stains.

Estimating an individual's biological age can be of great use when studying ageing or predicting disease susceptibility; however, estimating someone's chronological age would also be of significant value in criminal investigations. In cases in which there are no suspects or eyewitnesses available to provide investigative leads, predicting a bloodstain donor's age could eliminate potential suspects. There are various chemical or biological methods that have been proposed for age estimation in bones, teeth, or other tissues, such as lead accumulation, protein modifications, telomere length, and mitochondrial DNA (mtDNA) deletions; however, they all suffer from limitations including poor accuracy. Most of these methods are more likely to suggest an age group (generation) rather than accurately predicting age, which restricts their applicability in forensic casework.

Epigenetic analysis has been reported in the literature as an alternative or supplementary method for age prediction since DNA methylation of cytosines followed by guanines (known as CpG sites) is known to be one of the mechanisms responsible for cell differentiation and the cellular response to aging. Various genome-wide DNA methylation analyses investigating thousands of CpG sites at the same time have revealed a substantial decrease in global DNA methylation levels with advancing age. As a result, various age prediction models using a subset of these sites have recently been proposed for demonstrating good accuracy (average prediction error <5 years); however, due to the potentially low quantity and quality of crime scene samples, genome-wide approaches are not applicable in forensic genetics. Therefore, there is a need to develop methodologies that analyze only a few CpG sites without compromising accuracy and sensitivity.

In this study, hundreds of previously reported CpG sites were carefully selected from the literature as potential age-associated markers, and a dataset comprised of ~2000 whole blood samples was created using publicly available DNA methylation data. In an attempt to identify a subset of these markers to be included in the epigenetic-aging-signature, linear regression analysis was applied. Utilizing only the CpG sites showing the strongest association with age, an age-prediction model was generated using Artificial Neural Networks (ANN). The potential of ANN models in predicting complex characteristics has been previously explored showing great accuracy and reproducibility. Predictions were highly accurate for both the verification and blind tests. To apply this epigenetic aging signature in forensic casework, a protocol based on bisulfite conversion and sequencing using Illumina's® MiSeq® platform was developed and optimized using artificially made DNA standards of known methylation levels. Validation experiments revealed that the method is highly sensitive, accurate, and reproducible; therefore, the model's prediction accuracy was further investigated by analyzing a set of whole blood samples and mock casework samples. Following treatment with sodium bisulfite and amplification of the fragments containing the proposed CpG sites in multiplex bisulfite PCR reactions, libraries were prepared using an optimized NGS protocol. The resultant prediction accuracy was not as high as in the ANN model; however, it is believed that further optimization of the method could reduce prediction errors.

These findings provide a new quantitative tool for estimating chronological age in crime scene bloodstains, which, together with current methods, could provide new investigative leads in criminal cases. Future research will be able to expand on these results by identifying new markers, investigating population differences, or extending to different tissues.

Age Prediction, DNA Methylation, Next Generation Sequencing

B193 A Raman "Spectroscopic Clock" for Bloodstain Age Determination: The First Week After Deposition

Kyle C. Doty, BS, 2165 Robinwood Avenue, Schenectady, NY 12306; Gregory McLaughlin, MS, 100 Manning Boulevard, Albany, NY 12203; and Igor K. Lednev, PhD*, University at Albany, State University of New York, 1400 Washington Avenue, Albany, NY 12222*

After attending this presentation, attendees will better understand how Raman spectroscopy, coupled with 2D Correlation Spectroscopy (2D CoS) and chemometrics, can be used to analyze bloodstains, link known molecular changes with spectral variations, and predict the Time Since Deposition (TSD) for up to one week.

This presentation will impact the forensic science community by introducing a unique non-destructive method for confirmatory blood identification and bloodstain age prediction, accompanied by a statistical level of confidence, all of which could potentially be performed at the crime scene.

The identification of a body fluid stain is an important and necessary aspect of many forensic investigations. For blood in particular, knowing the TSD is highly desired in forensics, but it can be extremely complicated to accurately determine in practice. Although there have been numerous attempts to solve this problem using a variety of different techniques, currently no established, well-accepted method exists. Since the amount of suspected blood evidence may be miniscule, it needs to be preserved and analyzed efficiently. Therefore, a non-destructive method to competently identify human blood and predict the TSD would be highly valuable. Raman spectroscopy is a technique that has the potential for both non-destructive confirmatory identification of blood and for detecting molecular changes over time.

Raman spectroscopy has proven to be a versatile and effective analytical technique for numerous forensic applications, including the identification of drugs, explosives, gunshot residue, inks, paints, and dyes. Raman analysis often requires no sample preparation, is typically non-destructive, and has the ability to analyze microscopic amounts of sample. This technique is based on the detection of light that is inelastically scattered by a sample upon irradiation from a monochromatic light source. A Raman spectrum contains numerous distinctive bands that correspond to specific molecular vibrational modes. For blood in particular, Raman spectra provide rich detail and has been the subject of analysis in many forensic studies already. Popularity of Raman spectroscopy has been growing in forensic science, especially due to recent advancements in portable instrumentation and the breadth of both current and potential applications.

For this study, a Raman spectroscopic approach was developed for determining the age of bloodstains up to one week old. Raman spectroscopy, along with 2D CoS and statistical modeling, was used to analyze fresh bloodstains at ten time points under ambient conditions. The results of the 2D CoS indicate a high correlation between multiple Raman bands and the age of a bloodstain. A regression model was built to provide quantitative predictions of the TSD, with a cross-validated root mean squared error of 0.13 and an R^2 of 0.97. It was determined that a "new" (one hour old) bloodstain could be easily distinguished from bloodstains at other ages, which is very important for forensic science in helping to establish the relevant association of multiple bloodstains. Additionally, all bloodstains were identified as blood by comparing the measured spectra to multidimensional body fluid spectroscopic signatures. These results demonstrate that Raman spectroscopy can be used as a non-destructive analytical tool for discriminating between bloodstains on the scale of hours to days. This approach shows promise for immediate practical use in the field to predict the time since deposition with a high degree of accuracy.

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Raman Spectroscopy, Bloodstain Age, Chemometrics

B194 Investigations on the Use of Tissue MicroRNA Markers to Correlate Bloodstains With Wounds for Bloodstain Pattern Analysis

Donald J. Johnson, MS, California State University, Los Angeles, School of Criminal Justice and Criminalistics, 5151 State University Drive, Los Angeles, CA 90032; David Raymond, PhD, California State University, Los Angeles, Dept of Mechanical Eng, 5151 State University Drive, Los Angeles, CA 90032; and Ray de Leon, PhD, School of Kinesiology and Nutritional Science, California State University, Los Angeles, 5151 State University Drive, Los Angeles, CA 90032*

After attending this presentation, attendees will have an understanding of: (1) current limitations in bloodstain pattern analysis; (2) the probative value of wound tissue in bloodstains; and, (3) the effectiveness of micro RNA assays to identify trace quantities of wound cells in bloodstains and determine the wound-of-origin.

This presentation will impact the forensic science community by demonstrating that molecular markers can reveal information about the circumstances surrounding the deposition of blood evidence, namely the wound-of-origin. Otherwise indistinguishable and/or non-specific bloodstains can be further characterized by use of this technique. The implementation of the technique may enhance the work of forensic investigations and the administration of justice.

Certain bloodstain patterns can indicate the cause of the bloodshed. For example, an "arterial" bloodstain pattern indicates a breach to the arterial circulatory system; however, many patterns can be created by more than one mechanism. A pattern of several circular bloodstains on the floor, for example, could be the result of a minor injury or a life-threating/fatal wound. During the course of a homicide investigation, investigators may find blood from the victim or suspect, but the blood is found where one might expect to find victim's or suspect's blood for reasons other than the crime. The finding is further problematic in that the blood is in low quantities and in the form of a non-specific bloodstain pattern. The relationship of the blood to the crime then comes into question because of the circumstances of the case and the uninformative pattern of the blood. The blood may be evidence of the crime or the blood may be a coincidental finding unrelated to the crime. The finding may be used against a suspect or defendant, but the suspect/defendant may have a plausible alternate explanation for the finding; however, current forensic methods in this situation are greatly limited in the ability to test the different explanations and determine the correct circumstances under which the blood was shed.

In several homicide cases, evidentiary bloodstains with particular wounds based on the histological identification of wound tissue in the stains were able to be correlated; however, the finding of discernible pieces of wound tissue in bloodstains is rare in cases where the question is the bodily source of the stain, It is hypothesized that evidentiary bloodstains may contain trace quantities of wound cells, which can serve as markers to identify the specific wound or wound site from which the bloodstains originated and thus provide a means to answer questions as to the cause and significance of otherwise ambiguous blood evidence.

It has been reported that bloodstains can contain additional information about their origin in the form of wound cells. Using an animal model, bloodstains from a gunshot wound to the head were distinguished from bloodstains resulting from a gunshot wound to the chest by testing the stains for a brain micro RNA marker. The head shot and chest shot spatter patterns and the two sets of tested bloodstains were otherwise indistinguishable. Whereas proof-of-concept was achieved with the shooting experiments, the question then became whether the technique would be successful with bloodstains from injuries produced by less force; specifically, sharp force injuries. Hence, a proof-of-concept study was conducted on the use of micro RNA tissue markers to detect wound cells in blood drops resultant of stab wounds.

Specifically, investigations were conducted on the rat liver micro RNA marker, rno-mir-122-3p, with the QIAGEN® miScript® System and real-time Polymerase Chain Reaction (PCR) analysis. Intact rat carcasses were stabbed manually and with a mechanical device. Scalpel blades were used to stab the liver through the skin of the rat carcasses, and the blood on the scalpel blades tested positive for rno-mir-122-3p, whereas blood on the scalpel blade used to stab the lung through the chest wall tested negative for rno-mir-122-3p. Additionally, blood drops shed externally from the liver stab wounds tested positive for liver cells. The amount of the marker/cells in the stains appeared to be related to the sequence of the blood drops and the velocity of the blade. This research illustrates that molecular markers can reveal information about the circumstances surrounding the deposition of blood evidence, namely the wound-of-origin. The implementation of this technique may enhance forensic investigations and the administration of justice.

MicroRNA, Wound-of-Origin, Bloodstain Pattern Analysis

B195 Examination of Plastic Shopping Bags Using Attenuated Total Reflectance/Fourier Transform Infrared Spectrometry (ATR/FTIR)

Walter F. Rowe, PhD, George Washington University, Department of Forensic Science, 2100 Foxhall Road, NW, Washington, DC 20007*

After attending this presentation, attendees will understand how to obtain ATR infrared spectra from polymer films with high signal-to-noise ratios. Attendees will also understand the varied compositions of common plastic shopping bags and the capability of ATR/FTIR to differentiate shopping bags from different sources.

This presentation will impact the forensic science community by providing trace evidence examiners with knowledge of the composition of a common type of polymer material that may occur as forensic evidence. The presentation will also emphasize the value of paying attention to low levels of additives in polymeric products.

Plastic bags may appear in criminal investigations as packaging for many different types of evidence; however, only three publications have dealt with the forensic examination of plastic bags. Roux et al. analyzed a number of different types of bags (e.g., freezer bags, vegetable storage bags, sandwich bags, and general storage bags) purchased in Australia and in Asian countries.[1] Infrared spectrometry proved (along with visual examination) to be the most useful method for discriminating bags from different sources. Causin et al. examined shopping bags obtained from supermarkets in the Venice, Italy, area, using infrared spectrometry, thickness measurements, and differential scanning calorimetry.[2] The infrared spectra of the shopping bags fell into three categories: (1) pure polyethylene; (2) polyethylene containing calcium carbonate ($CaCO_3$); and, (3) polyethylene with a carbonyl stretch at 1740cm^{-1}. Hashimoto et al. analyzed plastic bags specially prepared by Japanese manufacturers using X-ray diffraction, infrared spectrometry and optical microscopy.[3]

This research used ATR/FTIR to examine shopping bags used in the United States and compare the results with previously published work.[1-3] Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) were applied to the data to see if these methods yielded greater differentiation of the spectra compared to simple visual comparison. Forty different plastic shopping bags were obtained in the Washington, DC, area from supermarkets, department stores, restaurants, and hardware stores. ATR/FTIR spectra were obtained from five different non-printed areas on each bag. The ATR/FTIR spectra were obtained using a minimum of four layers of bag material to insure high signal-to-noise ratios to facilitate detection of the peaks of minor constituents. The spectra were scanned from 525cm^{-1} to 4,500cm^{-1} at 4cm^{-1} resolution; 128 scans were collected for each spectrum.

The shopping bags were found to be homogeneous and comprised of polyethylene, usually with an inorganic filler. Most of the shopping bags were found to contain $CaCO_3$. As Causin et al. found in the shopping bags they examined, the concentrations of $CaCO_3$ showed considerable variation.[2] Two of the bags contained talc and one bag contained both $CaCO_3$ and talc. Talc and $CaCO_3$ were confirmed by X-ray diffraction.

The spectral data from 525cm^{-1} to 1,800cm^{-1} were analyzed by PCA. Prior to PCA, the ATR /TIR spectra were scaled by setting the absorbances of the most intense peak of polyethylene to the same value. Some of the scaled spectra had significant baseline offsets; these were removed by taking the first derivative of the spectral data. Finally, the first-derivative data were autoscaled prior to PCA. The first factor extracted by PCA represented the quantity of $CaCO_3$ present in the bags, while the second factor represented the quantity of talc. LDA was performed using the extracted principal components and placed the ATR/FTIR spectra in seven groups with 100% accuracy.

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Plastic Bags, Infrared Spectrometry, Principal Component Analysis

B196 X-Ray Powder Diffraction (XRPD) Method Development and Validation for the Identification of Counterfeit Pharmaceuticals

Mark R. Witkowski, PhD, U.S. Food & Drug Administration, Forensic Chemistry Center, 6751 Steger Drive, Cincinnati, OH 45237; Nicola Ranieri, BS, 6751 Steger Drive, Cincinnati, OH 45237; JaCinta Batson, MS, FDA's Forensic Chemistry Center, 6751 Steger Drive, Cincinnati, OH 45237 ; Lauren L. Richards-Waugh, PhD, Marshall University, 1401 Forensic Science Drive, Huntington, WV 25701; and Kelsey M. DeWitt, BS, 8 Pyramid Drive, Apt 822, Huntington, WV 25705*

After attending this presentation, attendees better understand the method development and validation for analyzing suspect counterfeit pharmaceuticals with the Bruker® D2 PHASER desktop X-ray powder diffractometer. Attendees will also understand how this method of analysis could be beneficial compared to other popular methods such as Fourier Transform Infrared (FTIR).

This presentation will impact the forensic science community by providing information on an additional method of analyzing and distinguishing counterfeit pharmaceuticals from authentic pharmaceuticals.

Counterfeit pharmaceuticals are illegally manufactured and widely distributed throughout the world, which is a major threat to public health. Counterfeit pharmaceuticals are unapproved and unregulated products which may contain dangerous or harmful ingredients or insufficient amounts of the Active Pharmaceutical Ingredient (API) the patients require to stabilize or improve their health.[1] Historically, counterfeit pharmaceuticals have been found to not contain the correct amount of API, to contain a different API, no API, or the incorrect excipients within the counterfeit product.[1,2] Fast, easy-to-use, and reliable techniques are required to screen and identify a suspected counterfeit product from an authentic product, ensuring the safety of the public's health.[2]

XRPD is a technique often used in forensic science to analyze various types of trace evidence. XRPD has been shown to be a useful technique in the analysis of suspect counterfeit pharmaceutical products.[3] Previous work has shown that the X-ray diffraction spectra of authentic products can be compared to those of suspect counterfeit products to differentiate authentic products from counterfeit products. In some cases, this technique can be used to determine the presence or absence of an API or other excipients within a dosage form, and ascertain whether the correct API is present within that dosage form.[1] This study describes the method development for analyzing authentic pharmaceutical solid-dosage forms, APIs, and excipients to be used to identify suspect counterfeit pharmaceuticals using the Bruker® D2 PHASER diffractometer at the Food and Drug Administration's Forensic Chemistry Center (FCC).

First, an XRPD spectral library was built by analyzing excipients and active pharmaceutical ingredient standards using the Bruker® D2 PHASER instrument. Next, authentic pharmaceutical dosage forms were analyzed and compared to the corresponding API and excipient standard XRPD spectra to determine if the standards could be observed within the dosage form pattern. The XRPD spectral variability between authentic tablets and different lots of a product were determined to assess the product changes between tablets and lot numbers of the product. Counterfeit dosage forms were then analyzed and compared to the authentic dosage form XRPD spectra to determine if the counterfeit products could be differentiated from the authentic products. The information provided in the United States Pharmacopeia (USP®) General Chapter <941> was used as a guide for the method validation.[4]

The APIs, excipients, and authentic pharmaceutical products chosen for the method development were based on pharmaceutical products for which the FCC has known examples of counterfeits. XRPD spectra of the APIs and excipients were collected first, then the authentic dosage forms were collected. A series of experiments were conducted to determine the optimum sampling and measurement parameters for the standard powders (APIs, excipients) and the dosage forms. The initial results showed that peaks in the XRPD spectra of the APIs were not easily distinguishable in the authentic dosage form XRPD spectra. This was determined to be attributed to the concentration of the API present in the dosage forms and the manipulation of the API during the manufacturing process. The manipulation of the API during the manufacturing process may change the crystallinity of the API in such a way that the XRPD spectrum of the API in the dosage form is different than that of bulk powder API. Based on this result, absence/presence of API could not be used to distinguish authenticity.

Instead of looking for the absence/presence of the API to determine authenticity, it was found that overall differences in the counterfeit formulation compared to the authentic product formulation could be used to determine authenticity. It was also determined that counterfeit products contain various excipients with different crystalline structures than the authentic product. In cases where the counterfeit tablet formulation was similar to the authentic product, the difference in the crystalline structure of the excipients in the counterfeit products resulted in peak shifts greater than 0.2° at the 2θ-diffraction angle for a given peak in the XRPD spectra. This would indicate a counterfeit product.[4] This method can be used to distinguish counterfeit pharmaceuticals from authentic pharmaceuticals by looking at the overall XRPD spectral differences (additional peaks, missing peaks, and peak shifts). This presentation will discuss the method development and validation work conducted at the FCC in using XRPD to differentiate counterfeit pharmaceutical products from authentic products.

The mention of specific products/instruments in this presentation is for information purposes only and does not constitute an endorsement by the Food and Drug Administration and/or the Forensic Chemistry Center.

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XRPD, Counterfeit Pharmaceuticals, X-Ray Diffraction

B197 Microextraction Capsules (MEC): A New Direction in Green Analytical and Forensic Sample Preparation

Abuzar Kabir, PhD, Florida International University, 11200 SW 8th Street, AHC4-215, Miami, FL 33199*

After attending this presentation, attendees will better understand the fabrication, working principle, and advantages of MEC in preparing different analytical, environmental, toxicological, pharmaceutical, food, and forensic samples for chromatographic separation and identification.

This presentation will impact the forensic science community by educating attendees interested in analyzing trace organic analytes in various sample matrices and also has the potential to offer a paradigm shift approach in sample preparation by complete elimination of time-consuming, error-prone, and labor-intensive sample pretreatment steps (e.g., filtration, protein precipitation, centrifugation, etc.) from the sample preparation exercises.

Following the sustained demand for establishing the principle of Green Analytical Chemistry (GAC) in all aspects of analytical processes, the current trend in sample preparation inevitably favors miniaturization of the extraction device to minimize sample volume requirement, to reduce or eliminate organic solvent consumption and to minimize the amount of waste generated in the sample preparation process. Due to the high consumption of toxic and hazardous organic solvents and other shortcomings in major sample preparation techniques, including Liquid-Liquid Extraction (LLE) and Solid Phase Extraction (SPE), a number of miniaturized and green sample preparation techniques such as Solid Phase Microextraction (SPME), Stir Bar Sorptive Extraction (SBSE), Thin Film Microextraction (TFME), Microextraction by Packed Sorbent (MEPS), Fabric Phase Sorptive Extraction (FPSE) have emerged during last few decades.[1-5] Among others, these techniques are environment friendly, do not require a high volume of samples, and are fast and efficient.

Despite all the advances in sample preparation technologies, most of the new generation sample preparation techniques cannot handle real-life analytical, environmental, toxicological, pharmaceutical, food, and forensic samples which often contain high volumes of particulates, debris, biomasses, and other matrix interferents and unavoidably require a sample pretreatment process (e.g., filtration, centrifugation, protein precipitation, etc.). Oftentimes, this sample pretreatment/cleaning step leads to significant analyte loss.

MECs are designed to completely eliminate the sample pretreatment/clean-up step from the sample preparation protocol.[6] MEC utilizes a porous tubular polypropylene membrane with 0.2µm pore size and a 5.5mm internal diameter to encapsulate sol-gel hybrid organic/inorganic sorbent in the form of monolithic bed or spherical particles. The porous membrane allows easy permeation of an aqueous sample containing the target analyte(s) while protecting the sorbent from being contaminated. A magnetic metal rod implanted into the MEC allows spinning of the device when placed on a magnetic stirrer and diffuses the sample matrix for fast analyte-sorbent interaction. Thus, high loading of sol-gel sorbent provides high sample capacity for target analyte(s), fast extraction kinetics due to the sponge-like porous architecture of sol-gel sorbents, and protection of the sorbent from contamination via encapsulation into a porous tubular membrane making MEC a formidable and robust sample preparation technique. After the extraction, a small volume of organic solvent can be used to back-extract the accumulated analyte(s). Due to the high preconcentration factor achieved in the sample preparation using MEC, no solvent evaporation or sample reconstitution is required. The prepared sample can be analyzed using gas chromatography, liquid chromatography, or capillary electrochromatography to obtain complementary information if a suitable solvent compatible with these chromatographic techniques is chosen.

Analytical data obtained from a number of real-life applications of MEC will also be presented showcasing its advantages, extraction characteristics, performance superiority, and analytical figures of merit.

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Microextraction Capsules (MEC), Sample Preparation, Green Analytical Chemistry

B198 Use of Time-of-Flight Secondary Ion Mass Spectrometry (TOF/SIMS) for Age Dating of Fingerprints and Spatially Resolved Quantification of Illicit Drugs on Fingerprints

Shin Muramoto, PhD, 100 Bureau Drive, Mail Stop 8371, Gaithersburg, MD 20899; Arian C. van Asten, PhD, Netherlands Forensic Institute, Laan van Ypenburg 6, The Hague, Zuid Holland 2497GB, NETHERLANDS; and Edward Sisco, MS, NIST, 100 Bureau Drive, MS 6431, Gaithersburg, MD 20899*

WITHDRAWN

B199 Characterization of Performance-Enhancing Peptides Via Ambient Ionization Time-of-Flight/Mass Spectrometry (TOF/MS)

Kyle E. Vircks, MS, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; Jesse M. Zavala, MS, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; Robert B. Cody, PhD, JEOL USA, Inc, 11 Dearborn Road, Peabody, MA 01960; Warren C. Samms, PhD, 1885 Old Spanish Trail, Houston, TX 77054; and Roger Kahn, PhD, Harris County Institute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will better understand how preliminary identification of proteins and other large biomolecules can be easily and readily attained using a high-resolution ambient ionization mass spectrometer, instrumentation that is becoming more common in forensic laboratories.

This presentation will impact the forensic science community by demonstrating methods for the analysis of various macromolecules in drug identification laboratories without the need for the specialized protein-sequencing systems typically required.

The increasing presence of recombinant Human Growth Hormone (rHGH) and related performance-enhancing peptides has been observed as internet vendors sell these substances not only to professional competitors but also to the general public. Most drug identification laboratories do not have protocols useful for the analysis and identification of such large molecules. The primary technique of Gas Chromatography/Mass Spectrometry (GC/MS) is limited to relatively small molecules that are readily vaporized at the inlet. This study investigated the feasibility of analyzing macromolecules by using a high-resolution TOF/MS coupled with an ambient ionization source.

Several methods for ionizing proteins prior to entry into the MS were investigated. Electrospray Ionization (ESI), which is common for ionization of such species, as well as Paper Spray Ionization (PSI) and, for simplicity, simple inlet ionization were tested. Mass determinations of various high molecular-weight peptides were successfully attained with all three ionization methods.

Proteins less than 10kDa were amenable to inlet ionization. This involves introducing a small aliquot of dissolved protein directly into the inlet of the MS with a glass capillary. Multiple charge states of the ionized protein were observed, similar to ESI, even in the absence of an externally applied voltage. The mass of the intact protein was calculated by deconvolution of the spectrum either manually or with spectral analysis software. The simplicity of this ionization method makes it especially well-suited for the analysis of small peptides in a typical forensic setting. In contrast, PSI involves spotting small aliquots of dissolved proteins on a triangular piece of filter paper. The tip of the filter paper is then positioned near the MS inlet while high voltage from the onboard voltage supply of the MS is applied to the opposite edge of the paper. Results of inlet ionization, PSI, and ESI approaches will be discussed.

To make it possible for large biomolecules to be further characterized beyond a parent mass, enzymatic digestion of larger peptides is also being investigated.

This project was supported by an award from the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this exhibition are those of the authors and do not necessarily reflect those of the Department of Justice.

Peptides, Mass Spectrometry, Ambient Ionization

B200 The Effect of Skin Debris on Gunshot Residue (GSR) Detection

Bryan R. Burnett, MS, Meixa Tech, PO Box 844, Cardiff, CA 92007-0844*

After attending this presentation, attendees will understand that skin debris (keratinized epithelial cells derived from the normal process of desquamation by the skin and skin oil) interferes with detection of backscatter electrons in the Scanning Electron Microscope (SEM) from particles on adhesive samplers.

This presentation will impact the forensic science community by showing GSR particles in an SEM analysis of adhesive hand samplers are missed due to overlying skin debris attenuating or blocking backscatter electrons.

GSR particles as large as ten microns are hidden by skin debris when viewed in the SEM by backscatter imaging with an acceleration voltage of 20kV or 30kV. A bleach solution made of sodium and calcium hypochlorite is used to effectively remove the skin debris revealing GSR particles on the adhesive sampler surface. The GSR particles are unaffected by this treatment.

There are two mechanisms for particle adherence on an adhesive SEM sampler. *Primary transfer* of skin debris and particles occurs while the adhesive surface of the sampler remains sticky. This has been noted to be approximately 30 dabs. *Additive transfer*, where particles accumulate on epithelial surfaces, starts with the second dab and increases with additional dabs. With more than 30 dabs, additive transfer predominates as a means for particle transfer from skin. GSR particles adhering to skin debris are removed from the samplers by the bleach treatment; however, there appears to be little risk of removal of the adhesive-entrained particles from the sampler surface by the bleach solution. The bleach treatment described here should only be used after analysis of an untreated sampler due to loss of particles that are adherent on epithelial cell surfaces.

It is apparent an automated SEM analysis at an acceleration voltage at 30kV of a GSR sampler will reveal more particles than at 20kV; however, particles still may be undetectable (below backscatter electron detection threshold) at 30kV due to the attenuation of backscatter electrons by overlying organic material. The bleach removal of the organics on a GSR sampler will reveal previously undetected particles, regardless of the acceleration voltage.

Hidden GSR may be present for a negative-result sampler due to covering by skin debris. It will take reports of actual case analyses (two analyses: before and after bleach treatment) to ascertain whether it is worthwhile to routinely perform the bleach treatment prior to an initial analysis.

Perhaps in that high-profile case, in which the initial analysis is not significant for GSR, an additional analysis with bleach-treated samplers will change that result.

Gunshot Residue, Skin Debris, Backscatter Electron Imaging

B201 Modeling of Elemental and Isotopic Data for Reference Populations Distribution Functions to Be Used in Comparison Evidence and Provenance Intelligence

Jurian A. Hoogewerff, PhD, National Centre for Forensic Studies, Faculty ESTeM, University of Canberra, Bruce - Canberra, ACT ACT 2601, AUSTRALIA*

After attending this presentation, attendees will appreciate the potential of using multivariate geochemical elemental and isotope data to establish background population data for comparison analyses of soil and other natural materials and the use of the spatial distribution of this data for geographical provenancing intelligence.

This presentation will impact the forensic science community by creating awareness that it is possible to produce fit-for-purpose population distribution functions for natural materials with existing geospatial data, which can be used in forensic comparison analysis and for provenance intelligence.

In many instances, comparison analysis of trace evidence involves statistically open sample sets (e.g., that the source of a questioned sample might either be in a control set or not). The extreme case is where no control samples are available. In such a case, any intelligence about a possible source of a questioned sample, or exclusion of non-relevant sources, would greatly assist an ongoing investigation as it could provide guidance as to where to deploy resources.

At first glance, using multivariate elemental and isotopic data, typically 30+ variables, would seem to enable high levels of evidentiary discrimination; however, due to the nature of physical and geochemical processes involved, many of the elemental and isotopic data distributions are not independent and thus typical Random Match Probabilities (RMP) are in the order of 1 in 1,000 to 1 in 100,000, which is much lower than in forensic nuclear DNA analysis where sets of typically 16 independent genetic markers give RMPs in the order of 1 in billions.

As with forensic DNA analysis, it is essential for any forensic or intelligence interpretation to make reasonable assumptions about the structure of the background population distribution (e.g., the Population Distribution Function (PDF)) for each of the variables, element concentration, or isotope ratio, in the relevant context of a case.

With closed sample sets, one can determine the PDFs by measurement, if affordable, and create a closed database. The database can subsequently be used in either a frequentist or Bayesian approach to determine the RMP and/or Likelihood Ratio (LR).

With open sample sets, the challenge is to extrapolate outside a (by its nature) closed measured control database or, as mentioned above, make predictions even without a control database. Although at first instance it may seem paradoxical to predict outside the "box," it is possible to take advantage of the aforementioned systematic physical and chemical processes and natural boundary conditions to model and predict the PDFs of element abundances and isotope ratios in natural materials. The models will have uncertainties but, as they can be quantified, their effect on the RMP and/or LR can be expressed. Typically those uncertainties are small relative to the magnitude of the RMP and/or LR and thus do not affect their probative value.

In this presentation, a combination of climate and geological data models for Europe will be used to create intelligence about the likely provenance of a murder victim from a cold case in the United Kingdom. This cold case is from the south of the United Kingdom where a beheaded and "behanded" torso was discovered in 1991 and buried as unidentified in 1994. Exhumation in 2010 and subsequent oxygen, strontium, and lead isotopic analysis combined with spatial models for these three isotopes gave the cold case investigators the information to reinitiate the search for the origin of the victim.

Distribution Functions, Provenance, Comparisons

B202 Forensic Pathology as a Forensic Science: What Is "Bias" and Why Does It Matter?

Andrew M. Baker, MD, Hennepin County ME, 530 Chicago Avenue, Minneapolis, MN 55415*

After attending this presentation, attendees will understand how forensic pathology — a medical discipline — differs from other forensic specialties and why "history" and "cognitive bias" are not the same thing. The scope of the forensic pathologist's obligations, which go far beyond simply performing autopsies, requires information from many sources to ensure that decedent identification, cause of death, and manner of death are correct. The breadth of this information, which forms the medical history, is sometimes confused with cognitive bias by those outside the medical profession.

This presentation will impact the forensic science community by providing forensic scientists, attorneys, and cognitive scientists with deeper insight into the practice of forensic pathology, giving them a working knowledge of the concepts of cause and manner of death and the methods by which these conclusions are derived. Forensic scientists will be much better positioned to understand, explain, and defend the practice of forensic pathology.

In 2009, the National Academy of Sciences (NAS) Report, Strengthening Forensic Science in the United States – A Path Forward was published. Though largely focused on broad issues spanning many disciplines, it is telling that the NAS assigned medicolegal death investigation its own chapter, not only identifying the challenges unique to this community but also recognizing that the mission of medicolegal death investigation is considerably different than the mission of other forensic disciplines. Many of the deficiencies and needs of the United States death investigation system cited in the report came as little surprise to the death investigation community, as they had appeared in previous reports spanning decades.

Among the forensic disciplines, forensic pathology is (almost) unique because it is the practice of medicine. Its methods are unlike those in other forensic specialties, its goals are different, its scope is often considerably broader, and the types of errors that may occur are unlike those in other forensic disciplines. In all cases falling under medicolegal jurisdiction, the medical examiner is responsible for ensuring the proper identification of the decedent, correctly diagnosing the cause of death, and properly opining on the manner of death. Though these tasks must be done correctly in every instance, the systems in which forensic pathologists perform their duties can run the gamut from ill-equipped offices that are little different from a 19th-century coroner to nationally accredited regional or statewide facilities employing board-certified forensic pathologists and state-of-the-art imaging and laboratory equipment.

In practicing their craft, forensic pathologists support many interests beyond the criminal justice system. Indeed, more than 90% of the sudden, unexpected, or suspicious deaths autopsied and certified by forensic pathologists are — at the end of a thorough investigation — found not to be criminal cases. Proper diagnoses and conclusions in these non-criminal cases provide closure for families, serve as powerful drivers for the allocation of public health resources and research dollars, and prevent the needless prosecution of crimes that never occurred.

Some in the jurisprudence and cognitive science communities have raised the specter of cognitive bias in forensic pathology. There is no *a priori* reason that forensic pathologists should be any more or less immune to cognitive biases than any profession. But forensic pathology's critics sometimes fail to recognize that legitimate medical and contextual history — the foundational basis of the practice of medicine — is not tantamount to inappropriate cognitive bias. Further compounding this misunderstanding is the occasional failure to separate the neutral term "cognitive bias" from pejorative accusations of incompetence, dishonesty, fraud, or corruption.

Manner of death — the medical examiner's classification as to the circumstances under which the decedent died and highly dependent on a variety of contextual data — is a vital component of death certification and has been a key metric for public health in the United States for more than 100 years. Other uses of the manner of death — in particular, in the courtroom — sometimes pose problems.

The optimal way to ensure quality work and defensible conclusions in forensic pathology, and to minimize bias, is to safeguard the independence of medical examiners through legislative or judicial guarantees. Medical examiners must be independent of law enforcement and prosecutors and available to consult with all parties in the criminal justice system.

Pathology, Bias, History

B203 Analyzing Linear Sequential Unmasking

Roger G. Koppl, PhD, Syracuse University, Dept of Finance, 721 University Avenue, Syracuse, NY 13244*

After attending this presentation, attendees will know how to apply decision theory to the evaluation of linear sequential unmasking.

This presentation will impact the forensic science community by demonstrating that linear sequential unmasking may go wrong if the crime laboratory is not an error-tolerant, high-reliability organization.

Linear Sequential Unmasking (LSU) has been proposed as a refinement of sequential unmasking to better limit bias and error in forensic science.[1,2] In the preliminary stages of analysis, examiners would be required to associate a degree of confidence with each determination made as their analyses unfold in real time. These confidence attributions are strategic choice variables for forensic examiners. The value of LSU is highly dependent on the organizational context, which influences the costs and benefits of different examiner choices. Confidence attributions may be informative or meaningless depending on the "error tolerance regime" created by the overall organizational context of the crime laboratory. High-reliability organizations accept that errors will happen and thus attempt to control the consequences of error through measures such as redundancy rather than responding to individual errors with blame and opprobrium.[3] As Rouse and Morris have put it, "Errors are not inherently unacceptable; however, it may be that the consequences of error are unacceptable. From this perspective, one may be able to tolerate errors as long as consequences can be controlled."[4] Linear sequential unmasking will not improve forensic science unless crime laboratories become error-tolerant, high-reliability organizations.

These results of decision theory throw into question linear sequential, which is a recent important proposal to combat observer effects and bias in forensic science. If the proposal is to have its intended effect of improving forensic science, it must be preceded by measures to improve error tolerance within forensic science. Thus, some of the emphasis and attention should be shifted from individual error and the cognition of individual forensic scientists and toward the organizational context in which they work. LSU will improve forensic science only if that context is improved. In particular, it will improve forensic science only if crime laboratories are error tolerant. The future of forensic science depends significantly on whether the forensic science community recognizes both the importance of the error tolerance regime within which individual forensic scientists work and the role of organization in shaping the error tolerance regime.

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Linear Sequential Unmasking, Bias, Error Tolerance

B204 Three Roads Converge: The Formation of the Houston Forensic Science Center

Daniel D. Garner, PhD, Houston Forensic Science Center, 1200 Travis Street, 20th Fl, Houston, TX 77002; Michael Grojean, PhD, Houston Forensic Science Center, 1200 Travis Street, Houston, TX 77002; and Amy L. Popejoy, MS, Houston Forensic Science Center, 1200 Travis Street, Ste 2552, Houston, TX 77002*

The goal of this presentation is to share best practices and lessons learned with other forensic science centers as they consider the pursuit of independence. This presentation's conclusions will also assist more traditionally structured forensic science centers as they adapt to shifting requirements, changing legislation, and an increasing demand for objectivity, quality, and timeliness of services.

This presentation will impact the forensic science community by illustrating the roles that culture, communication, leadership, and employee engagement play in the formation of a new forensic entity. Attendees will be able to replicate this approach for their own organizations to more effectively manage their changes.

The Houston Forensic Science Center (HFSC) was established in 2012 as a Texas local government corporation whose purpose is to provide the City of Houston and the surrounding region with independent forensic services. In 2014, HFSC took over control and management of the Houston Police Department's (HPD) Crime Lab, Crime Scene Unit, and parts of the Identification Division. This is the first significant transition in the United States in which a traditional extension of law enforcement reorganizes into an independent forensic body committed to sound business practice, scientific rigor, and strong performance orientation.

Subsequently, this also created a unique set of circumstances which generated substantial risk for failure. In practice, three of the riskiest activities are mergers/acquisitions, large organizational change, and significant culture shift. HFSC's formation contained elements of all three. Three separate systems converged: HPD classified officers, City of Houston (COH) civilian employees, and HFSC's newly hired employees. Personnel from the two established systems (HPD and COH) had strong cultures, long institutional memory, and a degree of uncertainty, sometimes even skepticism, about the new company. HFSC employees were new arrivals, largely joining after the company's formation and entering the ranks alongside HPD and COH workers. The three groups have differing benefit packages, labor contracts, promotion channels, performance systems, and management structures.

This presentation is offered as a case study on how to mitigate the risk of this type of organizational change, while using the diverse backgrounds and differing cultures to build strength and capability. Engaged management, open and ongoing communications, a workforce involved in the change, and a thorough examination of the culture needed for success had the greatest impacts on the process.

Organizational research suggests engaged management is critical to mitigate the risk of major change and culture shift. HFSC's board of directors is a balanced governing body that ensures the Center's objectivity and independence. The internal management and leadership team reflects the diversity of the company's three separate "feeder systems." Finally, HFSC's organizational structure is designed around eight forensic disciplines: firearms, controlled substances, biology, latent prints, digital, audio/video, toxicology, and crime scene, each with strong leadership.

For the new venture to succeed, both the legacy workforces and the new hires have to buy into the required organizational change and culture shift. The cornerstone is a shared vision to serve the justice system through independent, objective science, coupled with active and open communications. This included frequent company meetings to distribute information and solicit feedback, strong intra-discipline communication and a single-platform Information Technologies (IT) system. Further, staff were instrumental in the change process, participating in committees focused on planning, quality, and human resources.

Ultimately, all change must be firmly rooted in culture to have long-term viability and sustainability. Tools to measure the necessary culture for HFSC's success were developed to monitor, adjust, and shape the change and progress. This includes key cultural dimensions mapped onto "as-is" and "should-be" frameworks. Both qualitative and quantitative data were collected through focus groups, individual interviews, observational assessment (including crime scene ride-alongs), and surveys. Initial qualitative analysis identified keywords, concepts, and themes resulting in six broad values and 25 markers. The six values are professionalism, business focus, teamwork, agility, stewardship, and ethics. Subsequent quantitative collection and analysis allowed for the comparison of the culture that currently exists within HFSC and its ideal culture. These results then form the basis for allocation of attention, resources, and effort to areas that will have the greatest impact on culture shift.

Incorporation, Independence, Culture

B205 Embracing Change: The Challenges and Rewards of Transitioning From the Bench to Management

Julia A. Dolan, MS, Bureau of ATF, Forensic Science Laboratory, 6000 Ammendale Road, Ammendale, MD 20705*

After attending this presentation, attendees will better understand the challenges facing managers in forensic science laboratories and the differences in knowledge, skills, and abilities required by managers versus those needed by scientists. This presentation will also offer some recommendations to prepare for this type of career change and to improve the transition process.

This presentation will impact the forensic science community by providing a realistic view of the challenges faced by managers in forensic science and will provide an approach for laboratory systems to proactively address potential knowledge and skills gaps for their employees new to management and for those considering such a transition.

Most forensic science laboratories select their first-line supervisors and laboratory directors from personnel who have worked in their system — people who are good forensic scientists. One challenge with this model is that most bench scientists, having studied forensic science, chemistry, biology, etc., haven't had formal training in business, leadership, conflict resolution, or management. Although technical knowledge, skills, and abilities are an important foundation for forensic science laboratory leadership, these talents only represent a fraction of what is necessary to be an effective leader. Forensic science laboratory managers must also have the ability to communicate effectively in order to share workplace objectives and address personnel issues with subordinates as well as the ability to influence superiors in the organization regarding needs such as training, equipment, and personnel resources. Knowledge of the procurement process, how to effectively develop and defend budgets, how to motivate employees and address performance problems as well as myriad human resources and legal issues are all critically important for supervisors at all levels. Unfortunately, many forensic scientists are not provided opportunities to develop these skills prior to being considered for management positions. Of even greater concern, some new supervisors are not provided adequate support and training even after obtaining a managerial position.

Many of these challenges may be further exacerbated when a former peer is promoted from within to a supervisory position. Personnel issues can be more difficult to handle when a former peer is involved and the nature of pre-existing relationships have changed. Dealing with conflict resolution, performance evaluations, or poor performance can be problematic for seasoned leaders and even more so for new supervisors that were recently part of the peer group.

Other challenges include the constant need to do more with less and the increasing public scrutiny of forensic science. Infrequent, but highly publicized, cases of inadequate science or malfeasance on the part of forensic scientists have put all of forensic science in a defensive posture. This presentation will focus on some of the unexpected issues that a technical manager may encounter and how to prepare for them. It will also highlight some of the hidden benefits of providing leadership in today's forensic science laboratory.

Forensic Science, Management, Career

B206 Using Results-Based Data to Make Informed Management Decisions

Jenna L. Oakes-Smith, MFS, St. Louis Metro Police Department, 1915 Olive Street, St. Louis, MO 63103*

After attending this presentation, attendees will have a better understanding of how to make use of analytical result data to create and improve laboratory policies and management decisions. The data generated by a metropolitan police laboratory will be presented as a case study.

This presentation will impact the forensic science community by demonstrating how empirical-quality data can be used to focus and support management decisions. Using data that evaluates the quality of the evidence being submitted, managers can direct resources to evidence that will most likely yield usable results. Managers may also evaluate the effectiveness of their staff by using both quality and quantity metrics.

As the media continues to highlight the impact that forensic analyses can have on cases, the public and the courts are demanding more and more testing to be performed on evidence; however, as these requests increase, the resources needed to support them do not. Forensic laboratories are simply told to do more with less and to cut their backlogs at the same time that evidence submission is increasing. Intuitively, the logical choice would be to not process certain types of evidence routinely, but who makes those decisions and how can they be justified?

A laboratory must decide which types of evidence and which procedures yield the best results. This decision should not be based purely on analyst or management intuition, but rather on empirical, statistical data. By recording the results associated with particular evidence types, laboratories can decide how to prioritize evidence samples. Further, the same data can be used to evaluate the quality of work of an analyst. Are there trends in the data that shows that an employee consistently takes swabs that yield more useable DNA? Does one analyst have better results with differential extractions than another? Is there a crime scene technician that has a higher-than-average percentage of latent lifts with no value? By examining the data, laboratories can direct resources to training individuals to ensure that the evidence is collected and processed with the best available techniques. By focusing on the best evidence processed with the most effective methods, laboratories can ensure that they will get the most bang for their buck.

This presentation will provide the results of the St. Louis Metropolitan Police Department Crime Lab's DNA and latent sections as a case study for this data-driven management methodology. The laboratory began using their Laboratory Information Management System (LIMS) system two years ago to evaluate the quality of their analytical results. The results, as well as the related policy questions, will be presented.

LIMS, Management, Quality

B207 Applying the Queuing Theory in Forensic Cases Management

Khudooma S. Al Na'imi, MSc, Abu Dhabi Police General Directorate, Forensic Bio Sect, Forensic Evidence, PO Box 66722, Al Ain City - Abu Dhabi , UNITED ARAB EMIRATES*

After attending this presentation, attendees will understand some principles of the queuing theory.

This presentation will impact the forensic science community by introducing a key aspect of the queuing theory, which can be a means of more efficient forensic case management. This may accelerate the operation of a forensic laboratory and help take advantage of the available resources in a suitable manner, taking into consideration that there is less attention to the use of administrative principles in forensic sciences in general.

Processing more forensic cases is one of the challenging points in many forensic laboratories. This can result in performance and capacity reduction, formation of backlog, and unsatisfied customers (prosecutors, victims, the accused, and the courts). To be able to handle more forensic cases in an efficient manner, management queuing theory can be applied in different stages of the cases. There are many waiting intervals in forensic cases, such as before receiving, waiting in lab reception, in different laboratory processes, and so forth.

In queuing theory, "λ" is the Arrival Rate and "μ" is the Service Rate . The average number of cases (or evidence waiting to be examined) is $L=\lambda 2/\mu(\mu-\lambda)$. The average time cases or evidence spends waiting to be examined is $Wq=\lambda/\mu(\mu-\lambda)$. The probability that the laboratory is busy getting new cases or evidence is $\rho=\lambda/\mu$.

There are several queuing models according to the organizational situation such as: M/D/1 case (mean random arrival of customers, deterministic service, and one service channel-one examination); M/M/1 case (mean random arrival, random service, and one service channel); M/M/C case (mean random arrival, random service, and C service channel); and M/M/C/K case (random arrival, random service, and C service channels and K maximum number of vehicles in the system).

In this research, approximately 50 forensic biology cases were reviewed. The average number of customers or evidence waiting for service, the average time a case spent in queue, and the probability of services being busy was calculated using the above equations. The information and results were used to improve the work of the laboratory, better communicate with customers, and reduce the queuing time for cases. There is a need for further attention from the forensic community to the queuing theory in managing forensic cases, especially in a period of increasing services requests.

Queuing Theory, Case Arrival, Forensic Services

B208 Quality Assurance of the Biostatistical Workflow in Forensic Genetic Casework

Andreas Tillmar, PhD, National Board of Forensic Medicine, Artillerigatan 12, Linkoping SE-58758, SWEDEN; and Gunilla Holmlund, PhD, Rättsmedicinalverket, Artillerigatan 12, 58758, Linköping, AL 58758, SWEDEN*

After attending this presentation, attendees will understand the different components of a biostatistical workflow in forensic genetic casework. Attendees will also gain insight into different aspects of the quality assurance of such a workflow as well as become aware of the challenges in the validation process and the maintenance of personnel competence.

This presentation will impact the forensic science community by contributing to ideas for the design and quality assurance of a complete biostatistical workflow to be used in forensic genetic casework. This presentation will also illustrate how validation of this workflow can be carried out within an accredited laboratory.

Quality assurance is a key element in an accredited forensic laboratory. Quality involves several components such as: (1) validation of methods, instruments, and software; (2) documented maintenance; (3) secured chain of custody; (4) documented operating procedures; (5) traceability; and, (6) proven competence of the staff. The general goal is to produce test reports of forensic investigations with legal certainty.

DNA is a powerful tool in forensic analysis for linking a suspect to a crime scene, resolving biological relationships, and identifying disaster victims. Traditionally, DNA investigations can be divided into two parts: (1) the establishment of DNA profiles; and, (2) the evaluation of the evidential weight of these DNA profiles, given some hypotheses about the true circumstances. There are well-documented standards for quality assurance of the first part (for example, DNA extraction and DNA typing methods), but for the second part, guidelines have not yet been established within the community.

The evaluation of the weight of evidence, using a biostatistical workflow, includes several components such as different computational methods, reference data, secured transfer of case data, and expert opinions made by reporting officers.

Sophisticated biostatistical computation models are implemented in dedicated software packages making up the basis of the biostatistical workflow. To assure the quality of this workflow requires not only validation of software packages per se, but also validation of population reference databases and reference parameter settings. It also requires thresholds to be set for different verbal conclusions that are used in the final report, and it embraces qualification demands for the authorization of competence of expert practitioners. It also includes proficiency testing of the workflow as a whole.

A biostatistical workflow for use in forensic genetic casework has been designed, validated, and implemented. The workflow contains four different software packages and population reference data for more than 50 autosomal, X-chromosomal, and Y-chromosomal DNA markers from three different populations. Competence requirements specific for each software and type of casework have been implemented. The requirements also include maintenance of competence through annual exercises. In addition, parameters for validation of any changes in the workflow, such as a new version of a software, have been established to maintain continuity.

Quality Assurance, Statistics, Forensic Genetics

B209 Success Rates From Touch DNA in Property Crimes

Tammy Taylor, MS, Harris County Institute of Forensic Sciences, 9000 Almeda Road, #7304, Houston, TX 77054; Michael A. Donley, MS, 1885 Old Spanish Trail, Houston, TX 77054; Diana Gonzalez, MS, 1885 Old Spanish Trail, Houston, TX 77054; Nikia S. Redmond, MSFS, 1885 Old Spanish Trail, Houston, TX 77054; Katherine Welch, MSFS, Harris County IFS, 1885 Old Spanish Trail, Houston, TX 77054; and Roger Kahn, PhD, Harris County Institute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will be aware of the rate of success for obtaining useful DNA from touch DNA in property crimes.

This presentation will impact the forensic science community by providing data on touch DNA success rates from a laboratory with no limitations on case acceptance.

Genetic evidence left by a perpetrator at a property crime in the form of epithelial cells is often described as touch DNA. Touch DNA samples include any object that the perpetrator may have touched at the crime scene or any personal item left behind. These items include objects handled at the crime scene, tools used during the crime (e.g., flashlight or screwdriver) and clothing worn by the suspect. Items worn by the perpetrator have much higher levels of genetic material compared to other items that were briefly touched. The Harris County Institute of Forensic Sciences (HCIFS) Forensic Genetics Laboratory accepted all touch DNA evidence from property crimes from 2009 to 2015 without limit (i.e., no case acceptance policy was in place). The DNA results for touch and touch clothing DNA evidence from property crimes from 2009 to 2013 were categorized and analyzed to determine success rates as far as profile Combined DNA Index System (CODIS) eligibility and CODIS hits.

In total, the laboratory analyzed 4,548 property crime cases containing clothing and touch samples. Of these cases, 672 had clothing items left behind that were examined for the presence of DNA from the wearer of the item. Of these 672 cases, 435 (65%) produced a DNA profile suitable for entry into CODIS. Of the 4,548 cases, 3,876 cases had evidence touched by a suspect with 703 (18%) suitable for CODIS entry. The CODIS hit rate for both categories was similar with 53% (231 of 435) of clothing cases and 44% (309 of 703) of touch cases with CODIS hits. The offender hit rate in both categories was also similar at ~80%. Overall, 34% of the cases with clothing samples and 8% of the touch cases had some type of CODIS hit.

Despite the modest 8% hit rate, the number of touch cases submitted per year to the HCIFS Forensic Genetics Laboratory increased more than three-fold from 2009 (402) to 2013 (1,348) and, when polled, submitting law enforcement agencies strongly supported the continuation of touch DNA testing as the best opportunity to link cases and to identify possible perpetrators for burglaries and thefts.

Touch DNA, Property Crimes, CODIS

B210 Forensic Genetics in Brazil: A (Still) Brief History

Ana Paula S. Doval, Federal Police Department, Inc, Sais Quadra 07 Lote 23 - Sps, Brasília, Federal District 70610-200, BRAZIL; Meiga A.M. Menezes, MSc, Federal Police- Brazil, Sqsw 303 Bl B Apt 108- Sudoeste, Brasilia, Distrito Federal 70673302, BRAZIL; Guilherme Silveira Jacques, MSc, SAIS 07, Lote 23, Brasilia 70610-200, BRAZIL; and Helio Buchmuller, PhD*, SMPW 21, cj 2, lt 8, cs C, Brasilia, Distrito Federal, BRAZIL*

After attending this presentation, attendees will better understand how forensic genetics has been growing in Brazil and how this tool could help in reducing the threatening rate of 53,646 homicides and 50,320 rapes, estimated in 2013.[1]

This presentation will impact the forensic science community by presenting the history of forensic genetics in Brazil.

The history of forensic genetics began in 1986 in England. Since then, DNA testing has become optimized and standardized for its use as criminal evidence by the international community. Two years later, a paternity test using DNA was performed in Brazil by Sérgio Pena, in a non-official laboratory. Despite Pena's success, the first official DNA laboratory was not created in Brazil until 1994 at the Police of Federal District (Brasília).

In 2004, when only five of the 27 Brazilian states had official forensic DNA laboratories, the Ministry of Justice implemented a national policy committed to providing infrastructure, well-trained forensic scientists, and integration between states and the Federal Police (PF). The laboratory web then began to be woven. The next year, PF's DNA laboratory was set up and started promoting competency events for official forensic scientists from all over the country. The laboratory team made their first contact with Federal Bureau of Investigation (FBI) in the same year, intending to acquire the Combined DNA Index System (CODIS). They had also planned for the implementation of a quality system since the laboratory's inauguration. This was achieved through an **International Organization for Standardization (**ISO) 17025 consultation in 2008 and accreditation in 2014. Legal initiatives for a database creation and for legal writ of biological sample collection from sentenced criminals were also undertaken.

In 2009, an agreement was made between the FBI and Brazilian PF, allowing the use of CODIS (5.7.4 and 6.1 versions) in official laboratories. The 6.1 version was used for the second time in the world and the first time in a plane crash to support victim identification from the Air France 447 disaster. Experts from Brazilian PF identified all of the 50 bodies that were found using DNA, conforming to the International Criminal Police Organization (INTERPOL) proceedings, based on 430 reference profiles from relatives and direct references.[2] After the CODIS assignment, Brazilian states initiated the formal registration of their own databases. At the same time, national database conditions were provided. In 2012, Brazilian Congress passed a bill to collect DNA from those convicted of hideous crimes as well as from arrestees, under the conditions of a court order.[3] In 2013, a decree put 2012's law into power and provided national database creation, as well as the DNA Databases Network, a web that would link state, federal, and national databases.[4] Another two practical databases have been helping investigations in Brazil: (1) for missing persons from the military dictatorship period; and, (2) a national missing children and teenagers bank.

Forensic sciences are also alive at academic level. The Brazilian Academy of Forensic Sciences (BAFS) was conceived in 2011 during Brazil's candidature in the International Association of Forensic Sciences (IAFS) and was formally founded in 2012. Two conferences have been held since BAFS creation, promoting integration between research and applied knowledge for official laboratories.

In conclusion, this presentation provides a brief summary of forensic genetics development in Brazil and explains how DNA testing has been gaining space and strength at the judicial level.

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Brazil, History, DNA Database

B211 From Cold Case to Solved Crime

Lisa Mertz, MS, OCME, Dept of Forensic Biology, 421 E 26th Street, New York, NY 10016; and Krista Currie, MSc*, OCME, Dept of Forensic Biology, 421 E 26th Street, New York, NY 10016*

After attending this presentation, attendees will better understand the complexities and challenges faced in processing cold cases and will be presented with a case file example.

This presentation will impact the forensic science community by bringing to light the challenges faced in processing cold cases as well as the need to continue efforts to solve these open cases.

DNA testing has become the single most important forensic tool available in solving, prosecuting, and preventing crime; however, DNA processing techniques have only become well adopted and routine in aiding criminal investigations within the past 15 to 20 years. This is in part due to new technological advances in the field. With the implementation of new technology, cold cases are able to be re-accessed to see what may be possible in terms of examining evidence for biological fluids and/or biological material.

Investigations into cold cases face the same basic challenges as new investigations in terms of DNA testing, namely obtaining a DNA profile that can be uploaded to the database of forensic unknown samples to be compared to known individuals in order to solve the case. Still, additional complexities for cold cases exist. Cold cases may require testing on items of evidence that were unable to be processed at the time due to a lack of technology or due to obtaining an unsuccessful result. Processing a cold case involves greater cooperation among law enforcement, the crime laboratory personnel, and the district attorney's office. This is due in large part because simply locating the items of evidence to be processed can be both time consuming and challenging. Similarly, locating previous reports and previous DNA testing results can take great effort. From past reports, the crime laboratory can make a determination as to what DNA testing or additional testing is now plausible. After determining what is possible, challenges are faced with the processing of evidence that is old and may only result in the recovery of degraded DNA. Challenges are also faced in dealing with items of evidence that may have been unknowingly contaminated at the scene in a pre-DNA world in which there was less emphasis on proper protective equipment. Also, obtaining DNA elimination samples increases the challenge. A DNA profile may be able to be recovered on the item of evidence tested; however, obtaining elimination samples from witnesses or law enforcement personnel who may have come into contact with the item may be impossible.

Processing cold cases can be challenging not only in terms of the DNA processing, but also in obtaining funding and employing staff to locate, vet, and analyze these difficult items of evidence. Also, one must consider the legal limitations, as the statue of limitations may have already passed for these cases.

As a result of all the above-listed challenges, several cold cases remain unprocessed to this day and thus stay cold. A successful cold case will be presented. The challenges faced in this laboratory will be highlighted as well to showcase the need for the continued processing of these open cases.

Cold Cases, Unsolved, DNA

B212 National Institute of Justice's (NIJ's) "Using DNA Technology to Identify the Missing" Program: An Update

Charles M. Heurich, MFS, National Institute of Justice, Dept of Justice, OJP, 810 7th Street, NW, Rm 7204, Washington, DC 20531*

After attending this presentation, attendees will understand the NIJ's funding for solving missing persons and unidentified human remains cases. Attendees will also receive data on the impact of the program to date.

This presentation will impact the forensic science community by educating the community about an NIJ funding program that their agencies may not be aware of as well as the impact this program has had on solving cases.

The NIJ is the research, development, and evaluation branch of the Department of Justice. NIJ is the federal government's lead agency for forensic science research and development as well as for the administration of programs that facilitate training, improve laboratory efficiency, and reduce backlogs. The mission of the NIJ's Office of Investigative and Forensic Sciences is to improve the quality and practice of forensic science through innovative solutions that support research and development, testing and evaluation, technology, information exchange, and the development of training resources for the criminal justice community. Through the research, development, testing, and evaluation process, the NIJ provides direct support to crime laboratories and law enforcement agencies to increase their capacity to process high-volume cases and provide needed training in new technologies.

Based on a survey published by the Bureau of Justice Statistics in 2007, it is estimated that approximately 4,000 sets of unidentified human remains are found every year, 1,000 of which remain unidentified after one year. In addition, on any given day, there are approximately 100,000 people reported missing. Since 2003, the NIJ has initiated programs dedicated to assisting state and local law enforcement agencies and crime laboratories in solving missing persons cases and identifying human remains. In April 2005 at a national strategy meeting sponsored by the NIJ, former Deputy Attorney General James B. Comey spoke about the lack of uniformity in multiple Federal Bureau of Investigation (FBI) databases. As a result of the meeting, he directed the NIJ and the FBI to establish a national task force to assess existing databases and identify how to better encourage, facilitate, and achieve greater use of these databases. This task force recommended the creation of a new database which became the National Missing and Unidentified Persons System (NamUs).

This presentation will discuss the NIJ's "Using DNA to Identify the Missing" program, its goals and objectives, and show success stories associated with the program. The funding amounts, number of awards, and performance metrics, including number of samples tested, profiles developed, profiles uploaded to the Combined DNA Index System (CODIS), and DNA hits will all be presented. Successful project designs and several cases in which identifications have been made will be discussed. In 2007, the NIJ released NamUs to the public. The system is composed of three individual databases: a missing persons database, an unidentified remains database, and an unclaimed persons database. This presentation will also discuss some brief updates on the NamUs system, including future plans for the system.

Missing Persons, NIJ, DNA

B213 The Testing of Unsubmitted Sexual Assault Kits: An Update on the National Institute of Justice-Federal Bureau of Investigation (NIJ-FBI) Sexual Assault Kit Partnership

Gerald M. LaPorte, MSFS, National Institute of Justice, Office of Inv & Forensic Science, 810 Seventh Street, NW, Washington, DC 20531; and Heather E. Waltke, MS, 3601 Connecticut Avenue, NW, Unit 120, Washington, DC 20008*

After attending this presentation, attendees will have updated knowledge concerning federal support through the NIJ-FBI Sexual Assault Kit Partnership to reduce the number of Sexual Assault Kits (SAKs) waiting to be tested in the nation's law enforcement agencies.

The presentation will impact the forensic science community by conveying information the Partnership has garnered with regard to the collection, processing, and testing of SAK evidence from law enforcement agencies across the country.

The NIJ — the research, development, and evaluation agency of the United States Department of Justice — leads the nation in supporting the forensic sciences through research and by providing state and local crime laboratories with funding to help process and test evidence more efficiently in an effort to reduce backlogs. This presentation will provide an update on the NIJ-FBI Sexual Assault Kit Partnership initiative designed to help inform evidence collection, processing practices, and testing protocols for SAKs.

This research initiative began accepting SAKs from law enforcement agencies in September 2015 and has been helping to address a major need in this nation's forensic science and criminal justice communities to support state and local law enforcement agencies in their efforts to reduce the number of unsubmitted SAKs. The focus of this effort has been in the collection and analysis of valuable data concerning the nature of sexual assault evidence contained in previously unsubmitted SAKs. The effort has begun yielding knowledge related to the various processes associated with the intake of sexual assault evidence into the laboratory as well as with screening, testing, and analysis. An understanding of these processes is fundamental in the effort to inform best practices for collecting, analyzing, and testing evidence from sexual assault cases, particularly when faced with the processing of large quantities of unsubmitted SAKs. The knowledge garnered as a result of this continuing effort will be used to fulfill the long-term goal of improving current and future best practices for collecting quality evidence and processing SAKs in a more timely and efficient manner. This initiative directly supports the goal of carrying out analyses of samples from untested, unsubmitted SAKs so DNA profiles can be developed and placed in the National DNA Index System (NDIS). More violent crimes are solved as more DNA profiles are placed in the NDIS, which is considered one part of the Combined DNA Index System (CODIS), the Federal Bureau of Investigation's (FBI's) program of support for criminal justice DNA databases as well as for the software used to run these databases. The NDIS has been particularly helpful to investigations that are very old and no longer producing new leads. The Partnership has already begun generating CODIS hits throughout the nation, and the upload of hundreds of eligible profiles generated from DNA analysis as a result of this research provides direct aid in the investigation of violent crimes involving sexual assault.

Sexual Assault Kit, DNA Testing, Sexual Assault

B214 What Errors Are We Looking for and How Can We Look for More?

Charlotte J. Word, PhD, PO Box 5207, Gaithersburg, MD 20882*

After attending this presentation, attendees will better understand how different types of errors that may occur in a forensic science laboratory can be discovered and what steps may be taken to expand the detection of additional errors that occur during testing and reporting of test results.

This presentation will impact the forensic science community by providing information on the importance of error detection and the approaches that can be used to expand the recognition of errors, with particular focus on the detection of false positive and false negative results and conclusions.

It is human nature to make mistakes. It should come as no surprise that human errors occur in crime laboratories, and thus all accredited forensic science laboratories must have quality-assurance programs in place with procedures for the detection, evaluation, and resolution of errors. Technical errors, failures in the testing assay, and some types of contamination can often be detected through the use of positive and negative controls. Technical review processes can often prevent the reporting of some mistakes in the final laboratory report, such as misinterpreted data and typographical errors, through the independent re-evaluation of the data, interpretation, and conclusions. Proficiency tests are required for the routine monitoring of the laboratory test assays and for the ability of the analyst to correctly perform the testing procedure and report the test results and conclusions. Based on any findings of errors detected through these various processes, it is common practice for the laboratory staff to research the cause of the errors and to take corrective actions to ensure the appropriate test results are obtained and reported in an affected case. In addition, when the cause of the error can be determined, corrective actions can be put in place to improve policies, procedures, and practices in the laboratory to prevent future errors of a similar nature.

Many of these quality-assurance practices aid in the detection of errors that can be corrected prior to the reporting of the final test results and conclusions. But how effective are these procedures for detecting *all* errors that are made in a laboratory? Are there other practices that could be considered for the detection of additional errors that are now being incorrectly reported? Are there additional mechanisms that can be put in place in a laboratory to improve the recognition of false positive or false negative associations (defined here as the incorrect association of an individual to evidence from a crime scene and the failure to detect the association of an individual to evidence from a crime scene, respectively)? These questions will be the basis of this presentation, which will provide information regarding what errors are currently being looked for, the effectiveness of that self-assessment, and some suggestions of methods that could be employed to expand the evaluation of laboratory error and detection with the goal of providing the best services to the forensic science community.

Errors, Quality Control, False Positive

B215 The Proper Use of Standard Reference Material 2372 (SRM 2372) Human DNA Quantitation Standard for the Calibration of Commercial Quantitative Polymerase Chain Reaction (qPCR) Kit DNA Standards

Erica L. Romsos, MFS, 100 Bureau Drive, MS 8314, Gaithersburg, MD 20899; Margaret C. Kline, MS, 100 Bureau Drive, Gaithersburg, MD 20899; David L. Duewer, PhD, 100 Bureau Drive, Gaithersburg, MD 20899; and Peter M. Vallone, PhD, 100 Bureau Drive, Gaithersburg, MD 20899-8311*

After attending this presentation, attendees will understand why the National Institute of Standards and Technology (NIST) produces SRM 2372 Human DNA Quantitation Standard and how that standard should be used within an individual laboratory to calibrate internal commercial qPCR kit DNA standards.

This presentation will impact the forensic science community by bringing attention to the differences in commercial qPCR kits based on the lot of the DNA standard and the quantitation chemistry employed. The proper use of SRM 2372 will also be presented.

The NIST SRM 2372 Human DNA Quantitation Standard was produced to support the need for a human-specific DNA quantitation standard in forensic casework as a calibrant for commercially produced DNA standards. SRM 2372 is intended primarily for use in the value assignment of human genomic DNA forensic quantitation kit materials. SRM 2372 consists of three materials, one single-source male, one multi-source female, and one multi-source male/female mixture, all solubilized in TE-4 buffer.[1] The application of SRM 2372 is for calibration of commercial qPCR kit standards to aid in proper quantification of unknown DNA samples within a forensic workflow.

Commercial Short Tandem Repeat (STR) assays used by the forensic human identity community require tight control of the amount of sample DNA amplified in the Polymerase Chain Reaction (PCR). This requires the ability to reproducibly measure the concentration of human DNA in a casework sample extract prior to input in the PCR reaction. Approximately 500pg-1,000pg of input DNA will provide a balanced and an interpretable STR profile. Commercially available qPCR kits routinely are relied upon to determine the concentration of casework extract within forensic laboratories; however, assays employed rely upon commercial DNA standards for relative quantitation estimates.

Data shown will demonstrate the need for an SRM for the calibration of commercially produced qPCR DNA standards. This is due to an observed ~50% variation in measured DNA concentration between multiple lots of a commercially available DNA standard. This variation may lead a laboratory to possibly overestimate or underestimate the concentration of an unknown sample that could lead to an incorrect dilution within the PCR workflow. In addition, the variation in assigned concentration across several qPCR chemistries will be shown. The use of SRM 2372 is intended to enable the comparison of DNA concentration measurements over time, production lots, and within an individual laboratory. Additionally, manufacturers can use SRM 2372 to validate the values assigned to their own commercial qPCR kit DNA standards. Individual forensic laboratories should use SRM 2372 to validate the concentration of DNA qPCR quantitation kit standards and to verify the assigned concentration of an in-house or commercial DNA standard prior to use. This recalibration will result in decreased variability between varying lots of qPCR kit DNA standards. A specific example of how to properly use SRM 2372 to recalibrate a commercial qPCR kit DNA standard will be presented.

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SRM 2372, qPCR, Standard

B216 Is the Factor of 10 Still Applicable Today?

Simone Gittelson, PhD, National Institute of Standards and Technology, 100 Bureau Drive, MS8980, Gaithersburg, MD 20899-8980; and John S. Buckleton, PhD, PB 92021, Auckland , NEW ZEALAND*

After attending this presentation, attendees will have an updated empirical measure of the variability inherent in the assignment of a match probability.

This presentation will impact the forensic science community by showing results for the variation of match probabilities obtained using different allele frequency database data from the same ethnic group. The results from this study indicate that this variation can be larger than a factor of 10 when comparing match probabilities obtained for different populations from around the world.

The assignment of the weight of DNA evidence depends on a number of factors (allele probability estimates, the population genetic model used, the value of the coancestry coefficient, etc.). One of these factors is the allele probability estimates from a database. Key considerations include, but are not restricted to, representativeness and size of the database. The appropriateness of any particular database to any given case is a matter of judgment. This judgment is often based on relevant background information such as the location of the crime. It involves an unavoidable subjective element.

In 1996, the National Research Council (NRC) Committee on DNA report stated that, ‘‘…empirical studies show that the differences between the frequencies of the individual profiles estimated by the product rule from adequate subpopulation databases (…) are within a factor of about 10 of each other...’’.[1] This statement has proven valuable as a gauge on variability caused by the database; however, it was developed at a time before Short Tandem Repeat (STR) multiplexes and is overdue for an update.

The present study examines the validity of the “factor of 10” method for the interpretation of DNA typing results today. It compares the match probabilities obtained using hundreds of different allele frequency databases from around the world. More specifically, this study first simulated sets of genotypes from a database of interest based on the allele frequencies of that database. Then, match probabilities were obtained using the allele frequency data from the original allele frequency database used for simulating the set of genotypes. Similarly, match probabilities were obtained using allele frequency data from each of the other databases in the study corresponding to the same ethnic group. The match probabilities obtained using the data from the other allele frequency databases were then compared with the match probabilities obtained using the original allele frequency database used for simulating the set of genotypes. The results of these comparisons indicate variation among the match probabilities that is greater than a factor of 10.

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DNA Interpretation, Allele Probabilities, Subpopulations

DIGITAL & MULTIMEDIA SCIENCES

C1 Differential Forensic Analysis of Periodic Mobile Forensics Images

Mark D. Guido, MS, The MITRE Corporation, 7515 Colshire Drive, Mclean, VA 22102*

After attending this presentation, attendees will better understand applying comparison forensic images from mobile devices before, during, and after an event/mission for rapid and targeted triage.

This presentation will impact the forensic science community by demonstrating the research and results of an automated mobile forensic image comparison capability.

Differential analysis of forensic images or, in other words, the direct comparison of forensic images before, during, and after an event of interest allows forensic examiners the ability of avoiding analyzing a large amount of information that hasn't changed and instead focus on the data that has changed between images.[1] This has the effect of greatly speeding up analysis and intelligence gathering.

Periodic Mobile Forensics (PMF) is a research project that automates differential analysis to address some new-use cases: (1) mission hotwash — users control the starting point of the device, and compare the device at the end of the mission to gather data, identify usage, and assess whether the device was targeted or compromised during the mission; and, (2) travel to areas of concern — the device is used during travel to potentially hostile areas, when users want to assess whether it was targeted or compromised.

There are a few unique capabilities in PMF's approach to differential analysis. First, in the above used cases, PMF needs only to temporarily modify the device (sometimes even only after mission usage) and can reset the device back to the stock image, with no on-device software indicative of any modifications. Second, PMF is an automated differential analysis system, not based upon changes to files, but rather by blocks (bit runs) of data. PMF hashes the entire Negative And (NAND) storage by offset and only needs to collect the data that has changed on the device during the event of interest. The fact that PMF only needs to collect changed data speeds up collection, allowing PMF to potentially collect over bandwidth constrained mediums (such as mobile broadband), and allows PMF to immediately report on device integrity without having to address what file may have changed on the device.

PMF automates the generation of certain views into the collected data in support of differential analysis. PMF can generate a heat map of the changed data on each of the device partitions. PMF can gather audit information from the device during the usage period between the initial forensic image and the final forensic image, utilizing a series of forensic processes that target-specific and potentially important device usage information. PMF has the ability to target and visualize all added, deleted, or changed files and directories exclusively during the event of interest. These visualizations supplied during differential analysis help forensics examiners to quickly target the forensically significant data.

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1. Garfinkel S., Nelson A.J., Young J. A general strategy for differential forensic analysis. *Digital Investigation* 9 (2012): S50-S59.

Differential Forensics, Post Event, Image Comparison

C2 Using Deep Learning Methods for Forensic Image and Video Investigation

Zeno J. Geradts, PhD, Netherlands Forensic Institute, Laan van Ypenburg 6, Den Haag, SH 2497 GB, NETHERLANDS; and Arnout C. Ruifrok, PhD, Laan van Ypenburg 6, Den Haag 2497 GB, NETHERLANDS*

After attending this presentation, attendees will understand how to search through images based on deep learning methods.

This presentation will impact the forensic science community by illustrating how newly developed algorithms become available for use in forensic science and how they can be applied in casework. The possibilities and limitations will be discussed.

The amount of stored digital images and video material is growing very rapidly since the number of cameras are rapidly increasing, ranging from cameras of Closed-Circuit Television (CCTV) systems to smartphones, computers, and drones in combination with social networks. In complex crime cases or terrorist attacks, the number of images and videos that require processing are often too much to handle in a short period of time. Searching for a certain suspect or tracking persons in video images is often a challenge. To make the pre-processing for further forensic investigation more efficient, there are several approaches for assisting investigators with this process.

Deep learning techniques are now commonly used for searching through many images and videos. In this presentation, an overview is given of methods that are state of the art and which can help the forensic investigation with man-machine interaction. Due to increasing processing power, the deep learning techniques are more feasible to use, and searching in videos and images is easier; however, real-world images are often from different angles, have poor lighting, and other conditions are present that may make the retrieval more complicated.

In this presentation, several examples of deep learning are presented. Several computer vision techniques in combination are presented and the results for a database are also discussed.

More research in deep learning techniques is needed for optimizing the methods that can be used for searching quickly through many hours of video material and classifying the material by user-defined classes. One such example is the search of images of feet or hands that may be useful in child pornography cases in which suspects are only partially visible. Another interesting area is the example of an investigator searching for the brand and model of a camera based on images, where the images of known cameras and models are used as training materials. Another briefly discussed comparison is that of facial comparison.

Additionally, the presentation of retrieval results is a challenge, since most people can only actively search through image material for less than 30 minutes before they become tired and make too many mistakes in the retrieval process. More time is needed for forensic comparison, since it often requires a 1:1 comparison, which takes longer. A good pre-selection is important, and perhaps in the future, the results of deep learning methods can help in finding a likelihood ratio of a certain shape of a feature. Validation of these methods for use in forensic science is important; however, the current research is focused on how deep learning can assist forensic image and video investigation.

Deep Learning, Multimedia, Searching

C3 Discriminating Hacker Techniques by Individual Differences and Techniques of Neutralization

Gregory Bowen, University of Alabama, 4120 Ashington Drive, Birmingham, AL 35242; and Kathryn C. Seigfried-Spellar, PhD*, Purdue University, Computer and Information Technology, 401 N Grant Street, West Lafayette, IN 47907*

After attending this presentation, attendees will better understand the different hacker tools used to commit cyber attacks as well as their relationship to individual differences and techniques of neutralization.

This presentation will impact the forensic science community by being the first study to assess the relationship between hacker tools, individual differences, and techniques of neutralization.

According to Paganini, the top five cyber security threats are: injection vulnerabilities, buffer overflows, sensitive data exposure, broken authentication and session management flaws, and security misconfiguration.[1] In addition, Symantec reported an increase in "trojanized" software updates, malware, ransomware, and social media scams in 2014; in fact, there were 317 million new forms of malicious software created this past year.[2] It is clear that escalating cyber threats and vulnerabilities are a serious concern for both small and large organizations, as well as the private sector and general public.

The term hacking has evolved over the years, but in general it refers to the use of a computer to gain unauthorized access to information systems or to someone who exploits the vulnerabilities of computer networks.[3] Nonetheless, computer crimes are similar to other types of crimes, such as homicide, in that the same crime (e.g., unauthorized access) can be committed, using different tools (e.g., password cracking, sniffing), by offenders with different techniques of neutralization (e.g., denial of injury, denial of responsibility); however, the traditional focus on cybersecurity has been on the creation of better tools, rather than understanding from whom one is being protected.[4-6]

Overall, there are a variety of hacking techniques or tools; however, it is unknown whether individuals are more likely to use multiple hacking techniques (i.e., a generalist) or if they tend to focus on a specific method or tool (i.e., a specialist). In addition, it is unknown whether there is a relationship between individual differences and techniques of neutralization for the different hacking tools/techniques. Thus, the current study will be the first to assess the relationship between individual differences and the types of tools (e.g., a Distributed Denial of Service (DDoS) attack) used by computer hackers along with differences in neutralization (e.g., denial of injury).

This study will be conducted using an anonymous, internet-based survey created in Qualtrics, and respondents will be solicited from Amazon's® Mechanical Turk. Research studies have shown Mechanical Turk may be used to obtain high-quality data inexpensively and rapidly from a diverse participant pool and provides better generalizability than snowball sampling procedures.[7] The survey will assess the prevalence of current tools used to administer various cyber attacks (e.g., DDoS, password cracking tools). In addition, this survey will measure the respondents' personality characteristics and techniques of neutralization.

The results and future implications of the study's findings will be discussed.

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Hackers, Personality, Individual Differences

C4 Joint Test Action Group (JTAG) Tool Testing

Jenise Reyes-Rodriguez, BS, NIST, 100 Bureau Drive, Gaithersburg, MD 20899; and Richard Ayers, MS, 100 Bureau Drive, MS 8970, Gaithersburg, MD 20899-8970*

After attending this presentation, attendees will be aware of the importance of tool testing and will have better understand the JTAG tool-testing process conducted within the Computer Forensics Tool Testing (CFTT) project.

This presentation will impact the forensic science community by increasing awareness of the impact tool testing has on informing the forensic community of tool capabilities and limitations. Test reports provide a foundation for toolmakers to improve tools, help users make informed choices, and provide interested parties with an overview of any anomalies found. The presentation will provide an overview of tools capabilities for acquiring and analysis of data recovered from the memory of a mobile device using JTAG and various analysis tools capable of parsing JTAG binary images.

The CFTT project has been researching and testing forensic tools capable of acquiring and analyzing JTAG binary images. This presentation discusses all aspects of the testing process that are critical for producing a test report and the information reported by the analysis tools capable of parsing JTAG binary images.

A summary for the test results of the JTAG acquisition and analysis tools examined will be discussed for each JTAG binary image created for the following test cases: (1) acquisition — acquire mobile device internal memory using supported JTAG hardware/software; (2) subscriber/equipment-related data — review acquired subscriber- and equipment-related information (i.e., International Mobile Equipment Identity (IMEI), Mobile Equipment Identifier/Electronic Serial Number (MEID/ESN), Mobile Station International Subscriber Directory Number (MSISDN)); (3) Personal Information Management (PIM) data — review acquired PIM data (i.e., call logs (incoming, outgoing, missed), calendar entries, memos, Short Message Service (SMS), Multimedia Messaging Service (MMS) (audio, graphic, video), stand-alone files (audio, graphic, video), application-related data, social media-related data (Facebook®, LinkedIn®, Twitter®), internet-related data (browsing history, bookmarks); (4) deleted file recovery — review recoverable deleted data elements; and, (5) Global Positioning System (GPS) data — review data containing GSP longitude and latitude coordinates (routes, pictures, video).

Each test report contains an associated table comprised of two subcolumns that define a particular test category and individual subcategories that are verified when acquiring the internal memory for supported mobile devices within each test case. Each individual subcategory row provides results for each mobile device tested. The results are as follows: (1) as expected — the mobile forensic application returned expected test results — the JTAG tool acquired the contents and the analysis tool reported the data from the binary image successfully; (2) partial — the mobile forensic application returned some of the data from the acquired JTAG binary image; (3) not as expected — the mobile forensic application failed to return expected test results — the tool did not acquire or report supported data from the mobile device successfully; and, (4) Not Applicable (NA) — the mobile forensic application does not support reporting for a specific data element.

The presentation gives an overview of the CFTT process as applied to performing a JTAG acquisition and analysis of the acquired JTAG binary image.

The test reports are available from the Department of Homeland Security Cyber FETCH web site: https://www.cyberfetch.org/.

JTAG, Mobile Forensics, Digital

C5 Mobile Device Data Population for Tool Testing

Jenise Reyes-Rodriguez, BS, NIST, 100 Bureau Drive, Gaithersburg, MD 20899; and Richard Ayers, MS, 100 Bureau Drive, MS 8970, Gaithersburg, MD 20899-8970*

After attending this presentation, attendees will be aware of the importance of populating and documenting the internal memory of mobile devices in preparation for tool testing and will better understand the mobile device data elements and various data population techniques.

This presentation will impact the forensic science community by increasing awareness of the impact the tool testing process has on informing the forensic community of tool capabilities and limitations. Test reports provide a foundation for toolmakers to improve tools, help users make informed choices, and provide interested parties with an overview of any anomalies found. This presentation will provide an overview of techniques for populating and documenting the internal memory contents of mobile devices used for testing mobile forensic tools.

The Computer Forensics Tool Testing (CFTT) project has been researching and testing forensic tools capable of acquiring and analyzing mobile device forensic tools. This presentation discusses all aspects in preparation for testing tools critical for producing a test report.

Techniques for documenting and populating the internal memory for the following data elements will be discussed: (1) subscriber/equipment data — International Mobile Station Equipment Identity (IMEI), Electronic Serial Numbers/Mobile Equipment Identifiers (ESN/MEID), Integrated Circuit ID (ICCID), and Mobile Station International Subscriber Directory Number (MSISDN); (2) address book/contacts — contact name, number, and contact metadata; (3) Personal Information Management (PIM) data — databook, calendar, memo entries; (4) call logs — incoming, outgoing, missed calls, and call log metadata; (5) Short Message Service (SMS) messages — incoming, outgoing, drafts, and SMS message metadata; (6) Multimedia Messaging Service (MMS) messages — incoming, outgoing, drafts, picture, audio, and video MMS messages; (7) stand-alone files — graphic, audio, and video; (8) application-related data — native mobile device applications; (9) social media-related data — Facebook®, Twitter®, and LinkedIn®; and, (10) Global Positioning System (GPS) -related data — longitude/latitude coordinates for routes, checking-in, geo-tagged photos, and videos.

When testing mobile device forensic tools, it is advantageous to possess knowledge of the internal memory contents of the mobile device(s) used. Documentation of the internal memory contents provides the tester with the ability to determine if the forensic application is acquiring and reporting data completely and accurately. Techniques covered in the document are as follows: (1) email account data syncing: — populating the internal memory of a mobile device by pairing and syncing data contents (e.g., contacts, calendar entries, stand-alone files, etc.); (2) Wi-Fi data transfer — populating the internal memory of a mobile device with Wi-Fi capabilities over an internet-connected router; (3) Personal Computer (PC) synchronization — PC sync software provides the user with the ability to transfer data elements from a PC to the mobile device; and, (4) Bluetooth® data transfer — data transfer between two mobile devices that provide Bluetooth® data transfer facilities.

This presentation provides an overview of populating the internal memory of mobile devices in preparation for the CFTT testing process.

The mobile device data population setup guide is available from the CFTT web site: https//www.cftt.nist.gov/mobile_devices.htm.

Mobile Forensics, Digital, Tool Testing

C6 Defining, Measuring, and Mitigating Errors for Digital Forensic Tools

James R. Lyle, PhD, NIST, 100 Bureau Drive, MS 8970, Gaithersburg, MD 20899*

After attending this presentation, attendees will understand some of the limitations and constraints when trying to establish error rates for digital forensic tools.

This presentation will impact the forensic science community by increasing awareness in the community regarding error mitigation strategies that should be used instead of error rates to establish the reliability of digital tools. This presentation will also aid forensic practitioners in recognizing that asking about error rates of digital tools is asking the incorrect question.

Extraction of digital evidence from digital systems is dependent on software to interpret and present relevant data. The courts need assurance that any testimony based on software is scientifically sound and reliable. The *Daubert* guidelines list testing and establishing an error rate as two criteria for the court to consider before deciding admissibility of evidence in court.

In the context of *Daubert*, *error rate* has more the meaning of statistical Type I and Type II errors (i.e., rates of false positive and false negative decisions for matching questions such as: (1) is a sample from a suspect a match to a sample found at a crime scene?; and, (2) is a sample found at a crime scene a match to an item in a data base?)

These matching questions come up in several contexts (e.g., DNA, tool marks, finger prints, etc.). The answer to these questions can exclude a suspect from further consideration or identify a new suspect for closer investigation. Matching questions usually have a random component and can be treated as a statistical hypothesis test with false positive and false negative error rates that can be computed and stated.

These questions are also seen in some situations in digital investigations, such as using cryptographic hashing algorithms to determine if two files match. Hashing reduces an arbitrary length file, possibly quite large, to a short fixed length (128 bits for MD5, 512 bits for SHA3-512) hash value. Error rates can be constructed for hash algorithms: (1) if hashes differ, then the files differ; error rate is zero; and, (2) if hashes match, the chance that there is a hash collision (different files with the same hash) is 1 in $2^{n/2}$, where n is the hash length.

These error rates contribute to satisfying the question (of concern to *Daubert*) of the reliable scientific basis for using hashing to identify file matches; however, hashing illustrates that there are more issues to consider. For digital forensic tools, the implementation must also be considered. Errors in an implementation are not usually statistical in nature and are often triggered by a combination of non-random factors. Unlike elements of DNA within a human population that are stable and change slowly over time, the factors that are relevant to digital evidence change with the pace of technological evolution.

This talk will address how to define error for digital forensic tools, how to use tool testing to identify tool behaviors that are relevant to an investigation, and how to use the knowledge gained from tool testing to mitigate incorrect tool behaviors.

Digital Evidence, Software Testing, Error Rate

C7 Google® Chromebook™: Evaluation of Forensic Methods for Data Extraction

Marcus Rogers, PhD, Purdue University, 401 N Grant Street, West Lafayette, IN 47907; and Yoshitaka Takase, MS*, National Police Agency of Japan /Purdue University, 401 N Grant Street, West Lafayette, IN 47907*

After attending this presentation, attendees will be more familiar with the various forensic methods for extracting data for Google® Chromebooks™. The pros and cons of each method will be discussed.

This presentation will impact the forensic science community by providing information on data storage locations of Google® Chromebooks™ and how evidence can be located and preserved.

The number of Chromebook™ unit shipments has increased in recent years and this increase is expected to continue; correspondingly, more devices could potentially become objects of forensic examinations in various cases. Computers using Google® Chrome™ OS are highly dependent on being connected to the internet. Therefore, they usually contain a relatively small on-board storage capacity; the users are required to use the Google® Drive cloud storage. These systems also use primarily web-based applications.

The current research focuses on understanding the data extraction methods for Chromebooks™ in a laboratory or on site. Three empirical studies were conducted (using a typical case scenario) that focused on settings information and files related to the Chromebook™ device. The first study's objective was to determine the different settings information each user could show on the screen; the users were a Guest user and a Google® account user who was registered as an owner. It was determined that the latter user could show more information. The second study's objective was to determine methods for extracting files from the limited internal storage. The methods tested included manual extraction, designated file extraction, logical extraction, and physical extraction. It was found that manual extraction and designated file extraction were the best methods. Last, the research looked at the preservation of metadata and file integrity as a result of the file extractions from: (1) local drive; (2) cloud drive; and, (3) attached storage media. The results indicated that the "Files application" was a practical method for copying the files to an external drive attached to the Chromebook™, as the modified dates were not altered; however, when using Windows® Explorer or forensic software, the time stamp was interpreted differently. The time difference was the same as the time-gap from Universal Time Coordinated (UTC) (1-hour-gap between standard time and daylight savings time). The research concluded that with Google® Chromebooks™, manual extraction (e.g., taking screenshots, pictures, or notes) maintains the metadata the best and should be considered as part of any standard operation procedure for conducting forensic examinations and analyses of these devices.

Chromebook™, Digital Forensics, Electronic Evidence

C8 Case Study: Snapchat™ Picture Recovery From Mobile Device Unallocated Space

Joseph L. White, MS, US Army Criminal Investigation Laboratory, Digital Evidence-CFI, 4930 N 31st Street, Forest Park, GA 30297*

After attending this presentation, attendees will expand their general understanding of the value of utilizing multiple forensic tools and techniques to recover deleted graphical content from mobile devices, specifically those utilizing the Snapchat™ application, which is designed to not retain multimedia content.

This presentation will impact the forensic science community by providing an overview and example of picture recovery procedures utilized when recovering deleted Snapchat™ pictures from mobile devices utilizing multiple forensic analysis tools and techniques.

Forensic analysis of mobile devices is one of the most quickly evolving areas of Digital and Multimedia Sciences (DMS). With the development and release of mobile devices occurring at a very rapid pace, Digital Forensic Examiners (DFEs) and mobile forensic software companies are faced with the task of determining how to extract and interpret data from the constantly evolving hardware and software of mobile devices. As each new iteration of mobile device and/or mobile device Operating System (OS) is released, it must be determined how to not only extract data from the device, but how to convert the raw data into a format that makes sense to the end user. The use of mobile device applications, or apps, further complicates data analysis of mobile devices. Not only is the base OS of mobile devices under constant development, but individual application developers release and update apps at a surprising pace.

Snapchat™ is a mobile device application that allows users to send and receive multimedia content, such as pictures and video, between specified individual contacts. The transferred multimedia is termed a "Snap." Settings within the sender's Snapchat™ application determine how long the sent content will be viewable on the receiver's mobile device, from one to ten seconds. After the time limit has expired on the receiver's device, the content is allegedly erased. Security features of the Snapchat™ application are also designed to prevent users from taking screen captures of received content through other mobile device applications.

This presentation will discuss these issues through the results of an examination of an Android™-based mobile device submitted for examination to the United States Army Criminal Investigation Laboratory (USACIL) in a case involving the Snapchat™ application. The mobile device submitted to the USACIL belonged to an individual accused of soliciting nude photographs from underage girls through the Snapchat™ application. The accused admitted to sending and receiving content through Snapchat™, but insisted the received content did not contain underage nudity. The actual content of the Snapchat™ pictures became vital to the case. Joint Test Action Group (JTAG) data extraction resulted in a copy of the full device memory for analysis. Initial analysis indicated several snaps were received from the user names utilized by the young girls using the Snapchat™ application, but none of the content was viewable using the default settings of traditional mobile forensics software. Multiple forensic software packages were utilized in an attempt to recover the deleted content. Several illicit pictures apparently depicting the underage girls were eventually recovered from the unallocated space of the mobile device and provided for investigative agency review.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Snapchat™, Data Recovery, Digital Evidence

C9 Development of a Portable Mobile Phone Forensic Acquisition and Analysis Toolkit Utilizing Open Source Tools

Kelsey L. Wilkinson, BS, 1024 8th Street, Apt 5, Huntington, WV 25701; Robert J. Boggs, West Virginia State Police Digital Forensics Unit, 1401 Forensic Science Drive, Huntington, WV 25701; Joshua L. Brunty, MS*, Marshall University, 1 John Marshall Drive, Huntington, WV 25755; and Terry Fenger, PhD, 1401 Forensic Science Drive, Huntington, WV 25701*

After attending this presentation, attendees will understand the usefulness of a Raspberry Pi™ and open source tools for mobile phone acquisition and how to assemble a portable device using these tools.

This presentation will impact the forensic science community by demonstrating the possibility and effectiveness of analyzing mobile phones in a simple and affordable way. Using open source tools and a Raspberry Pi™ allows individuals to modify the device for their specific needs.

The Raspberry Pi™ was developed by The Raspberry Pi™ Foundation, a non-profit organization dedicated to educational charity. Since its release in 2012, the Raspberry Pi's™ use in the digital community has grown steadily. This small, credit card-sized computer allows people to develop and create their own projects and uses for the device beyond its intended concept of learning programming in the classroom.[1] Many forensics applications of this device have developed over the years as well, including penetration testing, surveillance, and network forensics.[2,3] The use of the Raspberry Pi™ 2 Model B to construct a small device with a touchscreen for mobile phone acquisition was researched. Using a Raspberry Pi™ and open source tools for acquisition could increase efficiency, while greatly lowering the cost for digital forensic laboratories.

Commercial tools have dominated mobile phone analysis in digital laboratories for years. Commercial tools are expensive and are not perfect — they can still miss data. In addition, some mobile devices are not supported by commercial tools. Open source tools are free and available to everyone; there is no need for licensing fees each year, which can cost a laboratory thousands of dollars. Since the programming script is open source, bugs or issues with the tool can be found and fixed quickly by users. Also, the open source feature allows examiners to modify and customize their forensic tools to their specific needs. The proprietary nature of commercial tools has made it difficult to explain and demonstrate the process of acquisition in court. With open source tools, the source code can be presented during trial.[4,5] A noted disadvantage of many open source tools is the use of the command prompt; however, some open source tools have added user-friendly Graphical User Interfaces (GUIs), such as Autopsy® (for Sleuth Kit®) or Development Environment For Tutorials (DEFT).

A simple device was developed for less than 300 dollars, utilizing both a 3D printed case and a small pelican case design. A ROBO 3D™ printer was utilized for the 3D printed version. An ARM7™-compatible operating system was loaded onto the Secure Digital (SD) card, and several open source tools with easy-to-use GUIs were tested for use with the device. Chosen open source tools were then compared to commercial tools for both Android™ and iOS® operating systems. The design, items, and development of the operating system used to create this device will be discussed in this presentation, as well as the results found during comparison studies. With further research and continued development of mobile phone forensic tools and GUIs, open source tools may prove to be a useful addition to digital forensic examiners' toolkits in the near future.

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Open Source, Mobile Device Forensics, Raspberry Pi™

C10 Forensic Analysis of Digital Audio File Structures and Formats

Catalin Grigoras, PhD, 1020 15th Street, Ste 8I, Denver, CO 80202; and Jeff M. Smith, MS, National Center for Media Forensics - CU Denver, 1150 10th Street, Ste 177, Denver, CO 80217*

After attending this presentation, attendees will better understand the process for building and deploying a database of audio samples for forensic purposes, specifically for use in the analysis of multimedia metadata, its structure, and characteristics of file formats.

This presentation will impact the forensic science community by disseminating results from the mass analysis of audio files in the Waveform Audio File Format Pulse-Code Modulation (WAV PCM) format produced/prepared by various recorders and software editors.

This presentation describes an extended study on the WAV PCM file structure and format analysis for forensic purposes. In conjunction with other analyses largely involving time and frequency domain measurements/plots, a framework for the authentication of digital audio includes analysis of the file structure and format as well as investigation of the suspected recording device itself.[1,2] Forensic audio is now commonly recorded as uncompressed .WAV files on small digital recorders and authentication of this evidence can end up being crucial in the courtroom. It is also common for digital audio recording systems that store data using a proprietary format and/or encoding to use custom software for conversion to WAV PCM. In the interest of authentication and establishing digital provenance of recordings, examples of traces left by different digital audio editors and converters will be presented.

The following table shows an example of the structure analysis results for four digital audio recorders from Alesis®, Olympus®, Marantz®, and Sony®, along with one Toshiba® audio converter. The preliminary results indicate that while some recorders and editors share the same file structure, other recorders and editors create files with additional metadata.

Alesis® PalmTrack	Olympus® DM-520	Marantz® PMD620	Sony® ICD-SX750	Toshiba® DMR-SX1
Ofs: 0 -> RIFF Ofs: 8 -> WAVE Ofs: C -> fmt Ofs: 24 -> data	Ofs: 0 -> RIFF Ofs: 8 -> WAVE Ofs: C -> fmt Ofs: 24 -> olym Ofs: 2D -> dss Ofs: 38 -> DM520 Ofs: 52 -> *timestamp* Ofs: 3F8 -> data	Ofs: 0 -> RIFF Ofs: 8 -> WAVE Ofs: C -> fmt Ofs: 24 -> bextZ Ofs: 154 -> *timestamp* Ofs: 386 -> data	Ofs: 0 -> RIFF Ofs: 8 -> WAVE Ofs: C -> fmt Ofs: 24 -> JUNK Ofs: 7F8 -> data	Ofs: 0 -> RIFF Ofs: 8 -> WAVE Ofs: C -> fmt Ofs: 26 -> data

Findings from an extensive study will be presented on the structure and format for WAV PCM files created by 31 commercially available digital audio recorders (example brands: Alesis®, Marantz®, Olympus®, Philips®, Roland®, SanDisk®, Sony®, Tascam®, Zoom®, etc.) and 20 processing and converting softwares (e.g., Adobe® Audition, DCLive Forensics, FFmpeg, Goldwave®, Olympus® DSS Player Pro, Sound Forge™, etc.). In addition to these findings, principles that can be followed in the collection and maintenance of reference sample databases for forensic analysis and how to use them in real cases when the suspect recorder is not available will also be shared.

Reference(s):

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2. Grigoras C., Rappaport D., Smith J. (2012) Analytical Framework for Digital Audio Authentication, *AES 46th International Conference*, Denver, USA

Audio Authentication, Multimedia Forensics, File Structure Analysis

C11 Proposed Analytical Framework for Electronically Frequency/Pitch-Modified Voices

Eliud Bonilla, BS, EB Technologies, LLC, PO Box 102, Kensington, MD 20895; Catalin Grigoras, PhD, 1020 15th Street, Ste 8I, Denver, CO 80202; and Jeff M. Smith, MS, National Center for Media Forensics - CU Denver, 1150 10th Street, Ste 177, Denver, CO 80217*

After attending this presentation, attendees will better understand the main challenges that electronically frequency/pitch-modified voices pose to speaker recognition efforts and the approach the National Center for Media Forensics is proposing in dealing with this issue in forensic environments.

This presentation will impact the forensic science community by providing a holistic analytical framework to use when dealing with electronically modified voices within investigative scenarios. Academic researchers and law enforcement personnel will also benefit since the framework will provide guidance in addressing a common core of questions.

The concept of the human voice as a reliable biometric signal is rapidly being accepted and implemented in today's society. State-of-the-art call centers are increasingly incorporating Automated Speaker Recognition (ASR) technologies in an effort to enhance customer service and minimize identity theft. In the forensics realm, ASR has been accepted in many European courts and may also be accepted in United States federal courts in the not-too-distant future; however, ASR is relatively fragile due to its dependency on the frequency dimension of the voice while not incorporating higher layers of information such as prosody and accents. It can be degraded by a number of inter-/intra-speaker characteristics, in addition to multiple variables along the signal chain.

Purposely modifying the frequency/pitch of a voice by electronic means is an effective counter-forensic measure. It is most often implemented to mask the identity of an individual. Its use can be considered legitimate when used to protect the identity of a witness in a television interview or as part of a law enforcement investigation; however, it can also be used to protect the identity of individuals committing crimes ranging from classic scenarios such as phone calls for ransom requests to recording video/audio messages inciting violence.

Electronic frequency/pitch modification impacts various common forensic analysis methods. Vowel spaces are distorted, thus neutralizing their use by phoneticians. Moderate changes degrade Likelihood Ratio (LR) scores in ASR systems while aggressive changes induce additional errors by distorting the format-frequency relationships outside of normal expected ranges.

The proposed analytical framework addresses four key questions that need to be answered in a satisfactory manner if the analysis is to have practical impact on investigative efforts. First, has the voice actually been electronically modified? Second, is the modification method or algorithm known? Third, are the settings of the method or algorithm known? Fourth, are the modifications reversible in order to enable conventional forensic voice comparison methods?

Attendees will be presented with a flowchart and guidelines that will facilitate incorporating future research in a holistic manner.

Voice Modification, Automated Speaker Recognition, Forensic Voice Comparison

C12 Age Estimation of Adolescents Using Eye Measurements From Various Angles in Videos

Neeka M. Parker, 932 1/2 12th Avenue, Huntington, WV 25701; Joshua L. Brunty, MS, Marshall University, 1 John Marshall Drive, Huntington, WV 25755; Robert J. Boggs, West Virginia State Police Digital Forensics Unit, 1401 Forensic Science Drive, Huntington, WV 25701; and Terry Fenger, PhD, 1401 Forensic Science Drive, Huntington, WV 25701*

After attending this presentation, attendees will understand how to use eye measurements to estimate the age of an individual in a digital video, even when the eyes are viewed from an angle rather than from directly in front.

This presentation will impact the forensic science community by providing information about how the angle at which an image of a face is taken can affect the measurements of that face and their usefulness in estimating age. This is particularly relevant when looking at pictures of people who appear to be teenagers and pre-teens, such as with potential child pornography. The appearance may be natural, but it may also be due to makeup, posing, clothing choice, or filter technologies available when taking a picture of oneself — also known as a "selfie." By relying on measurements of features of the face, including the eyes and pupils, rather than appearance age, the age of an individual can be estimated with fewer hindrances.

Due to the prevalence of social media and devices like smartphones, "selfie" photographs have become extremely popular. These and other images could be part of evidence in a criminal investigation. Any such images can be taken at any camera angle relative to the front of the face. If the age of the individual in the photograph is in question, it becomes necessary to have a way to estimate the person's age regardless of angle.

Institutional Review Board (IRB) approval was obtained in order to use human subjects. The target age group of participants was 11 years to 19 years old, with those less than 18 years of age needing documented parental permission and child assent while those 18 years of age and older only required documented consent. Due to the fact that the pupil is affected by many variables, including mood, medication, and lighting, images were taken under controlled conditions with the illuminance of the room documented. Participants were asked for their birthday and other general demographic information, as well as basic questions regarding mood, medication, and eye problem history. Photographs were taken using a Nikon® D3100 digital camera and Apple® iPad® iOS® Version 7.1.2. A set of two to four photographs was taken with the participants sitting 1.5 meters from the digital camera holding a forensic evidence ruler: one photograph was taken with the participant looking past the camera generating a spontaneous gaze and one was taken with the subject looking at the camera with an attentive gaze; an additional pair of photographs was taken if the participant wore glasses to produce images both with and without glasses. The participants were then asked to take a "selfie" and a short video using the iPad®, with the distance at which these were taken documented. Images were analyzed using Adobe® Photoshop® CS6, while videos were additionally analyzed using Adobe® After Effects®.

Nikon® images were analyzed and the data compared with the formulas given in MacLachlan and Howland.[1] Stills from the videos were analyzed to determine the angle relative to a reference point. The measurements made in these stills were analyzed for a connection to both the angle and the age of the participant.

Reference(s):

1. MacLachlan C., Howland H.C. (2002). Normal values and standard deviations for pupil diameter and interpupillary distance in subjects ages 1 month to 19 years, *Ophthalmic and Phsyiological Optics*, 22(3), 175-182.

Age Estimation, Visual Biometrics, Digital Video

C13 The Use of Photo Response Non-Uniformity (PRNU) Patterns for the Comparison of Online Videos on Social Media

Zeno J. Geradts, PhD, Netherlands Forensic Institute, Laan van Ypenburg 6, Den Haag, SH 2497 GB, NETHERLANDS; and Rick Cents, BS, Netherlands Forensic Institute, Laan, Den Haag 2497 GB, NETHERLANDS*

After attending this presentation, attendees will learn that camera identification with online videos should be well validated. In several instances, the wrong conclusion may be drawn by using this method.

This presentation will impact the forensic science community by illustrating how video compression may influence PRNU patterns for camera identification.

It can be important in a forensic investigation to determine the source camera which is used in the recording of specific movies; however, it can also be important to determine how many cameras are used in the recording of movies which are posted on social media platforms such as Facebook® or YouTube®. This research focuses on the use of PRNU patterns in online videos for the determination of the number of cameras used to record the given movies. PRNU is a type of noise present in a picture and video that is caused by the different reaction of pixels to light. Subsequent pixels should have similar values, but this is not always the case due to the manufacturing of the cameras. The PRNU pattern is used for camera identification and can be useful in, for example, child pornography or movie piracy cases. It can also be important to compare multiple online videos to determine how many cameras are used in the recording of specific movies.

Different cameras were tested to determine if it is possible to separate between movies recorded with the same camera and movies recorded with a different camera. The movies were first compared before they were uploaded to YouTube® and Facebook® and the results demonstrated that it was possible to distinguish between movies recorded with the same camera and movies recorded with different cameras. The videos were uploaded to YouTube® and Facebook® and were downloaded again to perform the same comparison. The results revealed that there was a difference in the PRNU pattern which was extracted from those movies. This was probably caused by the extra compression applied by the online platforms. The comparison between the movies demonstrated different results per camera. It was possible for the videos uploaded to YouTube® to distinguish between movies created with the same camera and movies recorded with a different camera when the Canon® Powershot® SX210 IS was used; however, it was not possible in two other models tested. The Canon® camera also provided the best results for the Facebook® videos, but 6 out of the 25 movies delivered an incorrect conclusion and this error rate was even higher in the other cameras. This shows that the compression applied by the online platform has much influence on the PRNU pattern. More research should be performed to optimize the current algorithms for the use of PRNU patterns in online videos.

PRNU, Camera Identification, Social Media

C14 Source Identification of High-Definition Videos — A Forensic Analysis of Downloaders and YouTube® Video Compression Using a Group of Action Cameras

Zac P. Giammarrusco, MS, 3338 Depew Street, Wheat Ridge, CO 80212; Catalin Grigoras, PhD*, 1020 15th Street, Ste 8I, Denver, CO 80202; and Jeff M. Smith, MS*, National Center for Media Forensics - CU Denver, 1150 10th Street, Ste 177, Denver, CO 80217*

After attending this presentation, attendees will understand how YouTube® video compression works and how it can affect different types of high-definition video analysis.

This presentation will impact the forensic science community by teaching investigators how to interpret and analyze video when the origins are on YouTube®.

This presentation addresses the effects of YouTube® on source camera identification while seeking to quantify the amount of change that can occur during the conversion process. It is well understood that YouTube® re-encodes all video uploaded to the site, which has several implications for forensic authentication analysis.[1] The testing material described in this study was comprised of 11 different GoPro® cameras and three different downloader tools.

Video cameras are a large part of today's mainstream society, where many people feel the need to record and share their life's experiences. YouTube®, created in 2007, has become the most popular host of internet videos from around the world with an estimated one billion unique monthly users.[2] YouTube® is localized in 61 countries and across 61 languages. More than 100 hours of video are uploaded every minute. These videos can contain important information about a crime, or event, that might have occurred. For example, in September of 2014, the terrorist group called the Islamic State of Iraq and Syria (ISIS) released a set of videos on YouTube® that portrayed the beheadings of American and British citizens. These videos were called into question, and their authenticity needed to be determined. It is the job of the forensic investigator to determine if a particular video in question is a complete and accurate representation of what it purports to be.

This research describes a variety of established image authentication techniques used to determine the origin of a video. The underlying framework of YouTube® is addressed, including how it works, and the effects it can have on a video in question. The research then describes and compares three tools that can be used for downloading YouTube® videos in addition to how the test data was acquired. The structure and source identification techniques are presented using the test results.

If a video is called into question, and a reference video database is available, the examiner can look for a match. In a forensic case, it is recommended that a database collected over time, with thousands of cameras and videos, be built to help determine the origin of a video. Since a reference population was available in this research, a threshold and a conclusion can be determined. This is the same principle that should be applied in all forensic cases.

The techniques discussed in this presentation are limited in providing positive proof of camera identification since the number of possible combinations between cameras, their settings, and eventually digital edits and recompressions before uploading to YouTube® or other video hosting services is almost impossible to compute. Due to the number of different variables, a set of conclusions is proposed that can be used within a framework for forensic cases.

Reference(s):

1. van Houten W., Geradts Z. Source video camera identification for multiply compressed videos originating from youtube. *Digital Investigation*, issues 1- 2, pages 48-60, September (2008).
2. www.youtube.com

Video Analysis, YouTube®, GoPro®

C15 The Authentication of MP4 Video Using File Structure and Metadata

Jacob R. Hall, 379 Mooney Hill Road, Patterson, NY 12563*

The goal of this presentation is to inform attendees about the file structure of MP4 video files, the contents of the multiple containers within each file, and the possibilities and limitations of using this information to authenticate an MP4 file.

This presentation will impact the forensic science community by showing a method of analysis to examine the meaningful components of an MP4 recording and parse them to identify the features of a recording that is consistent with an original recording from the device that was claimed to have created it.

With the widely available nature of smart phones and portable devices capable of recording high definition video, there is an overwhelming need for the ability to answer the basic question: Is this an original recording? Investigators have a responsibility to determine whether a file is authentic or if it has been edited in order to better approach their examination. Using the methods outlined in this presentation, an examiner can make a determination as to whether the file is an original recording. By examining both the structure of the MP4 file and the contents of its metadata, a meaningful decision can be made as to it's consistency with an original recording.

Using the QuickTime File Format specification, many important characteristics of a given file can be determined including the original device time and date stamps when the recording was made and the duration of the recording.

While the QuickTime structure contains a certain amount of useful information, there is a great deal of variability in how this data is stored between different device models and manufacturers. At its design level, there is an inherent need for certain data structures to exist in order to maintain files consistent with the QuickTime standard; however, these variations between device models and manufacturers present a unique opportunity for authentication based on a file's data structure and contents.

Using a collection of exemplar recordings taken from more than 20 devices, a baseline can be determined for how a given device stores the audio and video data. By comparing the file structures of these known original files to the file structures of files known to have been edited, differences in the structure of the file can be observed and shown to be indicative of a recording that is or is not consistent with an original recording. These inconsistencies in the files could include the structure of the file itself, the order and contents of the containers within the file, and the presence or absence of identifying keywords within the files metadata. If a given file has been edited in any way and then saved by a piece of third-party software, traces of this event will be evident in the resulting file.

Video Authentication, Mobile Devices, Forensic Video Analysis

C16 Challenges in Recovering Deleted Data in the Cloud

Robert Jackson, MS, SphereCom Enterprises, Inc, 7900 Sudley Road, Ste 416, Manassas, VA 20109; Richard Austin, MS, Hewlett-Packard, 5555 Windward Parkway, Alpharetta, GA 30004; Martin Herman, PhD, 100 Bureau Drive, MS 2000, Gaithersburg, MD 20899; P.W. Carey, MS, Compliance Partners, LLC, 250 S Grove Avenue, Barrington, IL 60010; and Otto S. Reemelin, MS, CBIZ, Inc, 3101 N Central Avenue, Ste 300, Phoenix, AZ 85012*

After attending this presentation, attendees will have a better understanding of the challenges faced by forensic investigators attempting to recover deleted data and metadata in cloud computing environments.

This presentation will impact the forensic science community by describing research performed by the National Institute of Standards and Technology (NIST) Cloud Computing Forensic Science Working Group, which was established to aggregate forensic science challenges in the cloud environment and to develop plans for measurements, standards, and technology research to mitigate those challenges that cannot be handled with current technology and methods. One of the highest priority challenges is recovering deleted data in the cloud.

Data deletion in the cloud is often based on the deletion of nodes pointing to information in virtual instances. Whether the deletion of the information has been fully achieved needs to be assessed and proven. Pathways for retrieval are dependent on cloud providers offering sufficiently sophisticated mechanisms for access. Recovery of data marked as deleted is difficult since it may get overwritten by another user in a shared virtual environment. The challenge becomes more difficult if the entire virtual environment is also deleted.

Issues in recovering deleted data in the cloud include the huge volume of dynamically and continually changing data; cloud resources previously assigned to the user may be unknown; deleted data are overwritten very quickly; there are multiple locations for any given data (data are moved around multiple servers and storage rapidly); there are likely to be multiple copies of data, leading to multiple deletions of data; and there is uncertainty about the proper owner of deleted data. Other issues include the geographically dispersed "incident scene" and the involvement of multiple organizations in multiple jurisdictions.

One important aspect is the role of end points. End points may contain file remnants, contact information, addresses, etc. that will assist investigators in identifying where the deleted data were stored or processed, and identifying other cloud resources that were provisioned; however, in many situations, such as criminal activity or cyber attacks, the end points that were used by the individual(s) involved will likely be inaccessible to investigators.

This presentation will describe the various characteristics dealing with data deletion in the cloud, the effects of various service and deployment models, technical requirements for recovering deleted data in the cloud, sample use cases that help highlight the challenging issues, and the role of standards and technology. For example, standards are needed for data retention (e.g., backups for live data, Virtual Machine (VM) snapshot images, audit trails, transaction logs) to allow easier development of forensics tools. From a technology perspective, forensic tools exist, but the biggest gap is the "needle in the haystack" issue (i.e., there is such a huge volume of data which is continually and dynamically changing that finding the deleted data, and then being able to attribute it to an individual, is a significant challenge).

Digital Forensics, Cloud Computing, Deleted Data Recovery

C17 Counterfeiting and Counterfeit Deterrence Applications for Imaging Technologies

Joel A. Zlotnick, MSFS, U.S. Department of State, 600 19th Street, NW, Ste 12.601, Washington, DC 20522*

After attending this presentation, attendees will better understand how imaging technologies are used by both counterfeiters and producers of security documents such as passports, visas, identity cards, and birth records, and how understanding the specific workflows used by counterfeiters can point the way to innovative counterfeit deterrence solutions based on the capabilities of imaging processes.

This presentation will impact the forensic science community by connecting the forensic science disciplines of questioned document examination and forensic imaging. As a result, attendees may find unexplored potential for greater cooperation in the areas of hardcopy counterfeit document deterrence and detection.

The forensic science disciplines of questioned documents and forensic imaging may be thought of as opposite ends of a spectrum in which hardcopy casework is in the realm of questioned documents and digital images are forensic imaging problems. Yet many common types of evidence in questioned document casework originate from a chain of analog or digital imaging processes, including (for illustration) the examples of trashmark comparisons for common source determination and examination of faxed or photocopied documents. Similarly, questioned document examiners routinely apply imaging techniques to resolve common casework problems. These include not just specialized techniques like electrostatic imaging for visualization of indented writing and alternate light source photography, but also more fundamental digital imaging techniques such as adjustment of contrast (and many similar enhancements) to scans or digital photographs of physical documents.

This presentation focuses on the relationship between the problems of counterfeiting and counterfeit document examination (usually regarded as questioned document issues) and the workflows used by counterfeiters to manufacture their products (which are unquestionably imaging processes). Counterfeiters possess two basic workflows through which they can manufacture fake documents: (1) a scan-and-print workflow where artwork is captured directly from a genuine document template and printed using process color devices; and, (2) a more involved artwork reorigination process in which the artwork is replicated, often using vector imaging tools, for printing using line art and spot color. The first option is popular with some counterfeiters because of its simplicity, but has limited ability to simulate the finer characteristics of genuine security documents. The second workflow has the potential to produce counterfeits that more closely approximate the artwork of a genuine document, but requires substantially greater skills and resources to accomplish.

This model is a significant oversimplification, since counterfeiters often blend these approaches. Further, it does not capture the complexities of simulating various classes of advanced document security features; however, it does provide a foundation for the idea that document artwork, by itself, plays an important role in counterfeit deterrence if it is used specifically to interrupt one of the two counterfeiting workflows described above. In fact, the important role of security document artwork in counterfeit deterrence is emphasized as sophisticated security feature technologies (such as optically variable devices and color shifting inks) become more widely adopted for non-security applications, which makes these technologies more accessible to potential counterfeiters and brings into question their value as standalone counterfeit deterrence solutions.

The basic techniques of counterfeit deterrence are, with certainty, rooted in imaging science. Certain security artwork strategies that are already in common use to combat one or both of the specific counterfeiting workflows described include the use of line art and spot color design, split fountain printing, microprinting, void pantographs, dedicated security halftones, and the use of inks encompassing expanded color gamuts (such as the use of Ultraviolet (UV) -responsive, metallic, or iridescent inks). These foundational security printing techniques are great examples of how inexpensive design strategies can make documents more resistant to counterfeiting; however, this presentation proposes that there is room for further work in this area and will describe some novel counterfeit deterrence concepts that specifically exploit differences between printing workflows used for production of genuine documents and counterfeit documents. The final purpose of this presentation is to initiate further conversation with the imaging science community regarding intractable forensic imaging problems, to determine if those problems can be purposefully extrapolated into the hardcopy printing world to deter counterfeiting.

Counterfeit, Imaging, Document

C18 H.Y.D.R.A. (Hyper Yield Data-Driven Real-Time Analysis)

Anthony Skjellum, PhD, Auburn University, Dept of Computer Science and Software Eng, 345 W Magnolia, 3101 Shelby Center, Auburn, AL 36849-5347; Austin Hancock, BS*, Auburn University, 3101 Shelby Center, Auburn, AL 36849-5347; Janice Canedo*, Auburn University, 3101 Shelby Center, Auburn, AL 36849-5347; and Erby Fischer, Auburn University, 3101 Shelby Center, Auburn, AL 36849-5347*

After attending this presentation, attendees will better understand a dynamic malware detection architecture for forensic science on Android™ devices that is also applicable to Linux®-based systems.

This presentation will impact the forensic science community by verifying and illustrating in detail the steps necessary to derive a data set composed of process control block variables that, when monitored in real time, are capable of identifying malicious behavior within milliseconds of its occurrence. This provides a dynamic architecture for forensic science on Android™ devices and supports live response as well as network defense and continuous monitoring.

Android™ and Linux® malware is an important class of threats to digital systems including mobile phones, tablets, Internet of Things (IoT), and portable devices. In this presentation, the focus is on advancements based on previous work that combines machine learning and process behavior to detect malware dynamically without resort to signature-based or other static methods. This research enables forensics studies of running systems.

Current static detection, including signature-based detection, fail to adapt quickly to the changing nature of malware in mobile devices. This inability to adapt quickly is inefficient at providing malicious behavior analysis, particularly in light of digital forensics. By utilizing a dynamic detection technique, attendees will overcome many of these aforementioned shortcomings. For example, the dynamic technique does not require familiarity with a given sample, knowledge of its signature, nor is it impeded by code obfuscation.

Research conducted recently by Dr. Farrukh Shahzad has shown that the execution behavior of a malicious Android™ application on Android™ 2.3 is markedly different from that of a benign Android™ application. To define execution behavior, a data set was determined by using a custom kernel module to monitor all variables in the Process Control Block (PCB) for an android process. Once a model of malicious behavior was obtained, it could be utilized in real time to classify an unknown application's behavior as malicious or benign.

This study intends to investigate the methodology demonstrated on the legacy system by Shahzad on current Android™ versions (e.g., Android™ 4.3, 4.4, 5.0) further. Dataset investigations from the legacy system will be demonstrated to determine the extent of changes vis-à-vis current Android™ versions. A comparison of the mathematics and methodologies utilized by Shahzad to both Bayesian Classification and Exploratory Data Analysis techniques will be analyzed to determine if they perform equivalently for malware detection.

For mining the data, system processes including core device processes and network performance are considered. Each system process is examined in an effort to find non-trivial correlations. For each correlation discovered, investigations are pursued further in an effort to prove a causation, since not all correlations lead to a causation. Both Exploratory Data Analysis (EDA) techniques and Bayesian classification techniques are explored in order to find causation, if present.

Both the validity and capability of alternative classification techniques on process behavior are covered. The work demonstrates the dynamic nature of the dataset as the Android™ operating system version progresses. The results from this investigation validate and refine a dynamic architecture for forensic science on Android™ devices.

Beyond Android™ and Linux®, the methodology created and advanced in this presentation can be applied in the future to other embedded and mobile operating systems.

Dynamic, Android, Malware

C19 A Comparison of Computer Forensic Tools: An Open-Source Evaluation

Adam Cervellone, BS, 611 22nd Street, Apt 323, Huntington, WV 25703; Robert Price, MS, North Carolina State Crime Laboratory, 121 E Tryon Road, Raleigh, NC 27601; Joshua L. Brunty, MS, Marshall University, 1 John Marshall Drive, Huntington, WV 25755; and Terry Fenger, PhD, 1401 Forensic Science Drive, Huntington, WV 25701*

After attending this presentation, attendees will better understand the capabilities of EnCase® Forensic 6, FTK® 5.6, and the SANS Investigative Forensic Toolkit (SIFT) Workstation 3.0, as well as learning if the SIFT Workstation 3.0 could be used as a viable forensic tool in a laboratory setting.

This presentation will impact the forensic science community by providing a clear and concise breakdown of the capabilities of the leading industry standard tools as well as a popular open-source tool. Very little documented research has been conducted comparing an open-source forensic tool with the industry standard tools; as such, this presentation will add to the research and hopefully encourage other studies.

The world of digital forensics is an ever-evolving field with multiple tools for analysis from which to choose. Many of these tools have very focused functions such as Mac® and iOS® device analysis registry examination, steganography analysis, mobile device examination, password recovery and countless others. Other tools are full-featured suites capable of analyzing a large case with multiple items.[1] The major problem with many of these tools is cost.[2] While they may be robust, they may not be affordable for a smaller laboratory that wants to engage in digital forensics.[3] This research focuses on industry standard forensic software such as: Guidance Software EnCase® Forensic 6, AccessData FTK® 5 as well as SANS' SIFT Workstation 3.0.[4-6] The SIFT Workstation is a freely available open-source processing environment that contains multiple tools with similar functionality to EnCase® and FTK®.[6] This study evaluates the processing and analysis capabilities of each tool. In addition to processing functionality, two other studies were conducted. The first is a virtualization study focusing on the ability of the software to create or help create a virtual machine from an E01 evidence file. The advent of cloud computing would make this an advantageous procedure in digital forensics.[3] The second is a simple cost analysis study. This portion of the research displayed how much a laboratory may have to spend to get a single examiner fully on-line with each tool. While comparison studies between commercially available software have been conducted and published, research comparing industry standard tools with an open-source tool is not well documented.[1]

For this study, mock test cases were created using North Carolina State Crime Laboratory (NCSCL) Mac® Minis and Dell® Latitude D810 laptops. The hard drives contained in these devices were hashed and imaged via EnCase® Forensic 6.19 and fully processed according to NCSCL guidelines in EnCase® Forensic 6.19, FTK® 5.6.3, and the SIFT Workstation 3.0. In addition to evaluating analysis, the tools were also evaluated based on their ability to create a virtual machine from the evidence file as well as on overall cost for a single examiner.[7-9]

This research has shown that the SIFT workstation is a viable option to use as a forensic tool, from a financial and functionality perspective. Its capabilities are vast and are similar to those of FTK® and EnCase® Forensic; however, due to its open-source nature and heavy reliance on the Linux® Terminal and command line, it is advised that only examiners highly skilled in Linux® use the SIFT Workstation for casework in order to maintain its viability.

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6. http://digital-forensics.sans.org/community/downloads.
7. http://forensicswiki.org/wiki/Virtual_machine.
8. Lesson 14-EnCase® Physical Disk Emulator (PDE) Module. In: *Guidance Software*. EnCase® Computer Forensics II. Pasadena: 2014; 173-185.
9. http://www.securityisfun.net/2014/06/booting-up-evidence-e01-image-using.html.

EnCase® Forensic, FTK®, SIFT Workstation

C20 Integrating a Profile of Frontal Face With Its Mirror Image for Facial Reconstruction

Paramjit Kaur, MSc, Panjab University, Centre for Systems Biology and Bioinformatics, Chandigarh 160014, INDIA; Kewal Krishan, PhD, Panjab University, Dept of Anthropology, Sector 14, Chandigarh 160 014, INDIA; Tanuj Kanchan, MD, Dept of Forensic Medicine, Light House Hill Road, Mangalore, Karnataka 575 001, INDIA; and Suresh K. Sharma, PhD, Panjab University, Centre for Systems Biology and Bioinformatics, Chandigarh 160014, INDIA*

After attending this presentation, attendees will understand the value of computer-generated programs in facial reconstruction when a half frontal profile of the face usually captured in Closed-Circuit Television (CCTV) cameras and other surveillance systems is available for forensic examination.

This presentation will impact the forensic science community by presenting a detailed analysis of the generated computer program, which will help attendees in understanding the concept of using this program for full facial reconstruction.

The face is an important part of the human body that is used to recognize and distinguish one person from a large number of people. This is due to the uniqueness of the human face and the great variability in the features observed in different human faces. Forensic anthropologists and scientists are interested in studying the various features of the face as this is useful for the reconstruction of a human face from an available cranium. Forensic anthropologists and scientists are also interested in establishing the biological profile of the person/deceased, such as estimation of age, sex, race/ethnicity as well as probable stature, in order to have the maximum number of clues for identification of the deceased. In this era of CCTV cameras, facial identification is based upon the comparison of the images obtained from a crime scene; thus, identifying human faces has become an important computer technology. At times with CCTV cameras and other surveillance systems, the complete face is not visible and only half of the frontal facial pose is captured; however, half of the mirror image of the face can be used to reconstruct the complete face of the person in question. In previous studies related to identification or in the field of computers, mirror images have only been used to solve the problem of a non-sufficient training sample and have not been used for full facial reconstruction.

In this presentation, a methodology is presented to generate a mirror image from one facial profile, merging it with the input image to generate a complete face. This procedure is very simple, less time consuming, and computationally efficient. The designed program was tested on a selected sample of five adult females ranging in age from 20 years to 30 years old. The photographs of the participants were taken against realistic backgrounds using standard procedures. The images were processed using IrfanView v. 4.38 software and a novel program was designed using MATLAB® (Version 7.9.0 (R2009B)). The program uses the right frontal profile of the participant's face as input and generates its mirror image. Finally, it merges both the input image and the mirror image to generate the complete face of the individual.

The facial asymmetry cannot be distinguished in the generated images because these images are the result of the mirror image formation of the right profile image of the face; however, even though the differences are discernable between the two types of images (i.e., the generated and the actual images), the faces are recognizable from the reconstructed images. Therefore, the present approach would help to generate a complete facial image in cases in which only one frontal profile (i.e., the left or right side of the face) are available for examination. The proposed methodology would also be useful for improving other facial reconstruction and recognition methods.

Facial Reconstruction, Computer Program, CCTV Captured Images

C21 Performance of Matching Algorithms in Non-Standard Expression-Variant Faces

Petra Urbanová, PhD, Masaryk University, Kotlarska 2, Brno, Czech Republic 611 37, CZECH REPUBLIC; and Igor Chalás, Masaryk University, Dept of Computer Graphics and Design, Faculty of Informatics, Brno, South Moravian Region 60300, CZECH REPUBLIC*

After attending this presentation, attendees will understand the basic principles on which mesh-to-mesh and deep learning algorithms for 3D face recognition are grounded. Attendees will also be more familiar with the performance of 3D-to-3D and 2D-to-3D matching algorithms as tested on expression-variant 3D recordings of human faces.

This presentation will impact the forensic science community by providing insight into current state-of-the-art forensic facial identification and by introducing approaches designed or applicable for processing of 3D faces.

The role of images (Identification (ID) photographs or surveillance videos) in personal identification is sometimes downplayed because image-based forensic evidence is, on many occasions, presented for examination in very poor quality, with non-standard framing, viewpoints, or under unspecified conditions. Furthermore, many images are unsuitable for automatic or semi-automatic face recognition systems due to non-standard poses, occlusion, or facial expressions. Development of 3D sensors, which has allowed recording depth information of human faces, has had a great impact on improving the robustness of facial recognition algorithms. Installations of 3D video surveillance systems and 3D capturing devices built for outdoor use have increased chances of securing 3D images at crime scenes and/or in the course of forensic investigations. 3D technology has been shown to compensate for many of the shortcomings traditionally associated with conventional 2D images and to improve performances of matching systems in personal identification if standard faces are processed; however, this technology becomes less successful if dynamic body features, such as hairstyle, facial hair, and, most importantly, facial expressions are incorporated.

Following previous studies, which indicated that matching 3D records of human faces outperform existing systems based on 2D images if tested on controlled expression-invariant faces, the present study sought to extend the objectives by exploring performance of matching algorithms using non-standard expression-variant 3D faces. The tested dataset was composed of an array of 3D faces with nine different facial expressions, including one with a neutral appearance, collected from 150 participants (for a total of 1,350 scans). For each individual, the array was acquired within one scanning session using the VECTRA® XT 3D imaging system.

Two matching algorithms varying in complexity and computation requirements were tested. The first falls under the rubric of 3D-to-3D image matching and was based on extracting 3D mesh geometry descriptors. The second applied 3D-to-2D conversion prior to computation and was based on a learning algorithm featuring convolution networks. For mesh-based matching, algorithms incorporated into FIDENTIS Analyst, a software application developed specifically for processing 3D faces, were tested. The program utilizes the Iterative Closest Point (ICP) algorithm for one-to-one, one-to-all, and one-to-average registration of 3D faces. Dissimilarity of aligned 3D faces is subsequently expressed via signed and absolute closest vertex-to-vertex distances. One scan per individual was selected randomly and tested against the FIDENTIS 3D Face Database (N~2,100 subjects; www.fidentis.cz). This was carried out repeatedly on the entire tested subset. A match/non-match decision was subsequently made while employing a classification model provided by linear discriminant analysis.

For the second approach, a deeper learning algorithm incorporated in CAFFE framework (caffe.berkeleyvision.org) was adapted. Prior to processing, 3D textured meshes were converted automatically into 2D color images (resolution 256 by 256 pixels) accompanied by corresponding depth maps using a simple in-house converter (Face viewer). In order to train the system, the dataset was split into training (75%) and testing (25%) subsets. To classify the images as matching/non-matching, the Berkeley Vision and Learning Center (BVLC) Reference CaffeNet classification model was employed. In both instances, performance of the tested algorithms was assessed by Receiver Operating Characteristics (ROC) curves (cross-validated by repeated random sub-sampling) and expressed in terms of Area Under the Curve (AUC), likelihood ,and odds ratios.

The results showed that both tested approaches were challenged by the presence of facial expressions in the tested data and their performance was poorer than when tested on 3D faces with neutral expressions; however, the deep learning approach proved that it could be very efficient if trained on a very large training dataset and, in a more user-friendly version, could be of help for forensic experts. Both tested algorithms were also shown to possess high demands on hardware and computation time. The work which deals with non-standard 3D images and departs from the recent trends of employing advanced computation and learning techniques may improve methods of facial recognition used in the forensic settings. The main purpose of this presentation is to present these prospects to the forensic community.

3D Face Recognition, Face Expression, Matching Algorithms

C22 On the Need for Social Contract Theory in the Ethics of Digital Forensics

Martin S. Olivier, PhD, University of Pretoria, Computer Science, Pretoria 0002, SOUTH AFRICA*

The goal of this presentation is to describe how professional codes of ethics are based on philosophical notions of ethics, with deontic and consequentialist theories most often seen. Aristotelean virtue ethics are also encountered. This presentation will also indicate that none of these theories provide the subject of a digital forensic examination with sufficient protection. By eliminating these theories and considering the fiduciary duty of digital forensic examiners, it is argued that an ethics theory based on social contract theory best balances the interests of the examiner and the (innocent until proven guilty) examined.

This presentation will impact the forensic science community by indicating that an ethics theory or code of conduct for a forensic examiner not only depends on what one expects from an ethical forensic scientist, but also on the nature of the artifacts examined. The artifacts examined in digital forensic science are often of an intimate nature and deserve to be examined with special care. Social contract theory provides a widely accepted basis to balance the interests of the various parties in an examination. It is hoped that these insights will lead to the establishment of a code of conduct for digital forensic examiners that reflect the special nature of examined artifacts.

The conclusion of this presentation is that a code based on social contract theory is not only indicated by the argument provided above, but will also assist in providing practical moral guidance. It is argued that such a code will necessarily be one that is not a mere list of good practices, but one that provides principles that need to be applied (or interpreted in a given situation) to provide moral insight.

Professional ethics typically manifest as normative guidelines describing the proper conduct expected from professionals. Professional status stems from extended education and training in a specific discipline that permit an individual to execute specialized tasks in society, where members of the society, due a lack of similar skills, have no option but to rely on the work of the professional. Codes of conduct are derived from various ethics theories with (professional) duty, (utilitarian) fairness, and notions of professionalism (virtues) usually all present to a greater or lesser extent. Such codes typically also include some requirements of allegiance to the profession as well as submission to sanctions by professional bodies. Codes worth exploring for such examples range from ancient texts, such as the Hippocratic Oath, to more modern codes in the forensic science domain, with prominent examples being those of the American Academy of Forensic Sciences (AAFS), the American Society of Crime Laboratory Directors (ASCLD), the Global Information Assurance Certification (GIAC), and the SANS Institute.

Distilling professional codes of conduct to their bare essence usually yields two elements: (1) the need to act with integrity; and, (2) the need to act to the best of one's ability where the ability is expected to be at a very high level — a level that justifies the professional epithet. This presentation argues that this second requirement is insufficient for forensics, in general, and digital forensics, in particular.

The basis of the presentation's thesis is the fact that forensics is a family of applied sciences. Ethics in science is a topic that has been studied from multiple perspectives: impact of research on participants; potential scientific bias of researchers (due to commercial, authority-related, gender-based, and other influences that the researcher may be unaware of); and the expected behavior of the scientist. This presentation takes its primary cue from this third category. Again, once distilled, it is clear that the primary demand on the scientist is to act with integrity; however, the paper argues that there are subtle differences between the expectations of integrity in the scientific and professional contexts. The forensic scientist has to conform to both flavors of integrity. Finally, the subject matter of the digital forensic scientist adds a third flavor of integrity that constrains his or her actions.

Above, the phrase "flavor of integrity" was used to imply that the concept of integrity — nebulous as it may be — remains the same; however, what one emphasizes about it may differ from instance to instance. The (Platonic) ideal form of science is one that searches for the truth above everything else. Hence, integrity in a scientific endeavor refers to choices that seek the truth above all else. Note that scientific integrity does not require the achievement of this ideal (which is impossible), but a dedication to seeking truth. At first glance, the dedication to truth seems that an imperative approach to forensic ethics is appropriate; however, the German philosopher Immanuel Kant showed that autonomy is required for a deontic approach. While the forensic scientist should not be constrained in seeking the truth, this scientist is bound by science — hence, total autonomy is not an option. Similarly, seeking truth is a virtue that suggests an Aristotelian approach; however, Aristotle's focus on a golden mean is inappropriate. Similarly, balancing outcomes in consequentialist theories renders utilitarianism impractical.

This presentation suggests that the difficulty in finding a home for forensic science in the best-known classical ethics theories stems from the fact that forensics potentially exerts control over the individual by helping to determine guilt or innocence (keeping in mind it is not only the guilty who stand accused of wrongdoing) and this power may be sovereign. This suggests a social contract theory as a key element in determining the appropriate ethical behavior of forensic science (and, ultimately, the forensic scientist). Given that John Rawls's seminal work already straddles constraint of power through the social contract theory and the domain of ethics, this approach is an obvious theory here.

Forensic power increases in the case of digital evidence. While forensic science makes truth claims related to human beings, everyday digitalization causes digital forensic power to permeate the individual's essence. This is cause for the clear balance of power and purpose.

Professional Ethics, Digital Forensic Artifacts, Social Contract Theory

C23 An Efficient and Effective Forensic Analysis Approach for the Internet of Things (IoT)

Anthony Skjellum, PhD, Auburn University, Dept of Computer Science and Software Eng, 345 W Magnolia, 3101 Shelby Center, Auburn, AL 36849-5347; Ankit Kumar Singh*, Auburn University, Dept of Comp, Sci and Software Engineering, 3101 Shelby Center, Auburn, AL 36849; and Janice Canedo*, Auburn University, 3101 Shelby Center, Auburn, AL 36849-5347*

After attending this presentation, attendees will better understand an IoT Forensics Framework that consists of both device and network-level forensics.

This presentation will affect the forensic science community by providing direction toward addressing changes in digital forensics with the introduction of IoT devices. Identification of the limitations of existing, early models for IoT Forensics with a new, resource-conscious paradigm offered for IoT Forensics combines concepts from continuous monitoring (network forensics) and computer forensics.

The IoT is an emerging distributed network of billions of smart devices ("things") that possess the ability to communicate and exchange data. The number of such devices is expected to increase rapidly, resulting in generating massive amounts of data. Considering these factors, Digital Forensic (DF) investigations will face new challenges arising from the ubiquitous use of the IoT in society. DF investigation processes including identification, collection, organization, and presentation in the context of the IoT devices must be understood, planned-for, and recognized as significantly different than the processes for common devices such as smart phones, tablets, servers, and Personal Computers (PCs).

Existing procedures used for handling DF investigations aren't sufficient for the IoT. Some investigators have already recognized this. For example, one recent model for the IoT-related crime investigations, Forensic Aware IoT (FAIoT) described by Hasan et al., requires that all registered IoT devices be monitored and that they store potential evidence in a shared repository.[1] A second model, the Forensic Edge Management System (FEMS) advanced by Oriwoh et al., proposes the use of a smart device that would be used for real-time monitoring and forensic services within a Smart Home IoT environment.[2]

While current models focus only on a device-driven architecture, this study's strategy is to create an IoT Forensics Framework that includes device and network forensics. The IoT devices will be connected and communicate through a network; therefore, identifying forensic elements and retrieving relevant evidence from the network is vital. To group similar events efficiently, investigative techniques are covered using data mining techniques including Bayesian Classification.

Overall, a systematic analysis approach geared toward handling IoT-related forensic investigations is needed. An IoT Forensics Framework is proposed that will impact all stages of forensic investigations and make the IoT domain more forensic-ready. Systematic trade-offs of static and dynamic resource overheads will be shown in order to achieve sufficient degrees of forensic fidelity. An argument that all IoT Forensics is best implemented as continuous monitoring, includes forensics for devices as well as the associated networks, uses secure protocols for communication between FEMS smart devices and other devices, and has small events grouped as super events shall be made clear to the attendees.

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Internet of Things, Forensics, Framework

ENGINEERING SCIENCES

D1 Richard III Discovered: The King's Remains

Sarah V. Hainsworth, PhD, University of Leicester, Dept of Engineering, Leicester LE1 7RH, UNITED KINGDOM; Guy N. Rutty, MD, University of Leicester, Forensic Pathology Unit, Robert Kilpatrick Bldg, Leicester LE2 7LX, UNITED KINGDOM; Jo Appleby, PhD, University of Leicester, Archaeology & Ancient History, Leicester LE1 7RH, UNITED KINGDOM; and Alison L. Brough, BS, University of Leicester, Forensic Pathology Unit, RKB, Leicester Royal Infirmary, Leicester LE2 7LX, UNITED KINGDOM*

After attending this presentation, attendees will understand how modern forensic techniques used for tool mark analysis can be applied to determining how injuries were sustained to a 500-year-old skeleton.

This presentation will impact the forensic science community by demonstrating how techniques from forensic engineering science, forensic pathology, and archaeology have been combined to understand the wounds and weapons used to cause the injuries found on the King's skeleton.

Richard III was King of England between 1483 and 1485. In 1485, King Richard III rode with his army from Leicester to fight against Henry Tudor, later crowned King Henry VII of England. The battle was fought at Bosworth Field approximately 20 miles from Leicester. Bosworth was a short battle lasting approximately two hours. The battle reached its end when Richard was killed.

Of all the monarchs of England since 839, Richard III was the only one whose final resting place was unknown. One legend had it that his body had been thrown into the River Soar and was lost forever. Other tales told of a church ruin with a plaque on a column claiming to mark his grave.

In 2012, the University of Leicester was given permission by Leicester City Council to excavate a site — a council car park that, from scrutiny of ancient maps, could well be the site of the Greyfriars church where Richard may have been interred.

Despite limited funding and more than 500 years of lost records, the very first trench dug in the car park exposed a skeleton. Two further trenches revealed the outline of the ancient church and confirmed that the skeleton was buried in the choir of the church, a mark of high status. Moreover, the skeleton showed pronounced scoliosis, a curvature of the spine, and evidence of battle injuries.

Carbon dating revealed that the skeleton was of the correct age to be Richard III. Mitochondrial DNA testing and a Bayesian statistical analysis demonstrated a conservative estimate of 99.999% confidence that the skeleton was indeed that of Richard III. The results of the find were announced by the University of Leicester to worldwide media coverage in February 2013. In the United States, this find is often referred to as "the King in the Car Park."

There were 11 injuries to the skeleton: 9 to the skull, 1 to the rib, and 1 to the pelvis. Ten of the injuries were considered peri-mortem while the pelvis injury was most likely a postmortem injury. Three of the injuries to the skull showed evidence of striations from tool marks and tool mark analysis determined that these marks were likely made by the same blade.

This presentation will exhibit the computed tomography and micro-computed tomography images that were taken from the skeleton and explain how the injuries relate to weapons from the period.

This presentation will be relevant to forensic engineers, pathologists, and anthropologists who have an interest in tool mark analysis and the relationship between tool marks and weapons.

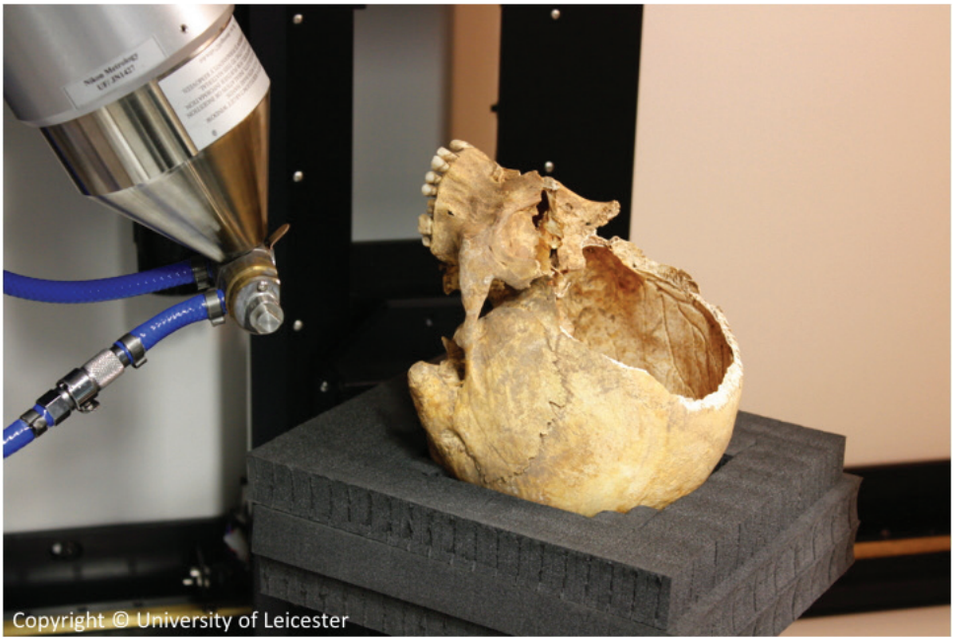


Figure 1: The skull of Richard III mounted in the micro-computed X-ray tomography scanner.

Richard III, Microcomputed X-Ray Tomography, Tool Marks

D2 Measurement of High Temperature and High Humidity Moisture Effects in Football Helmet Elastomeric Energy-Absorbing Padding Performance and Implications for Head Injury Danger

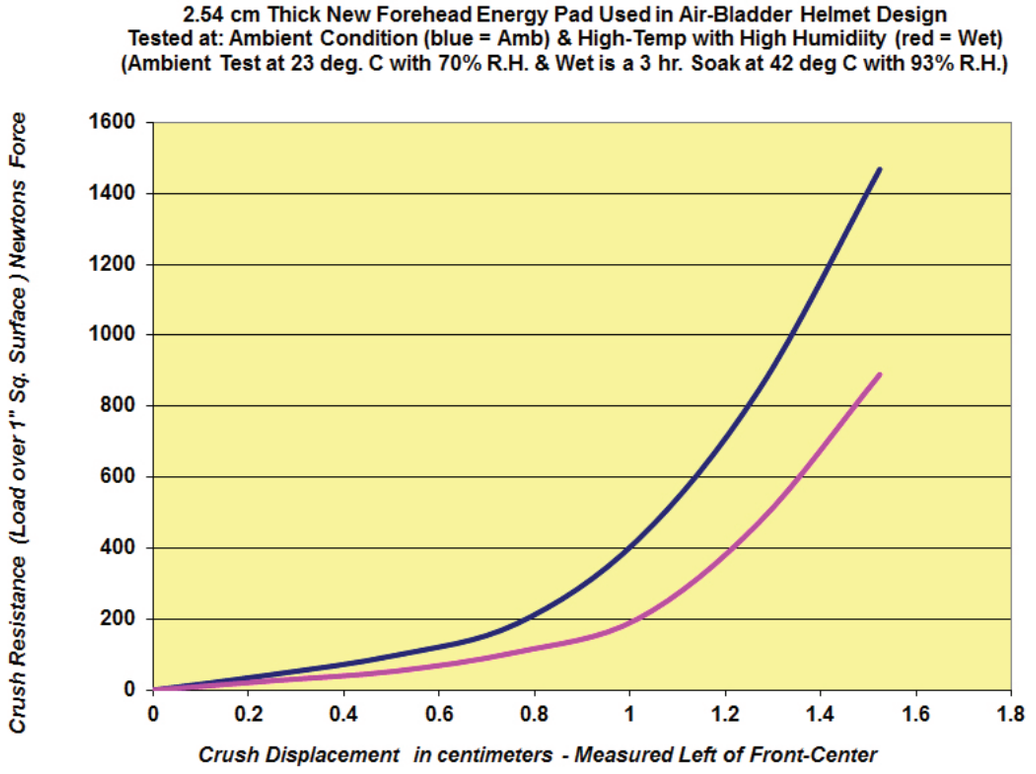
Kenneth J. Saczalski, PhD, 1440 W Bay Avenue, Newport Beach, CA 92661; Mark N. West, BS, Environmental Research & Safety Technologists, 1440 W Bay Avenue, Newport Beach, CA 92661; Todd Saczalski, BSMET, 140 Calle Irena, Sedona, AZ 86336; Joseph L. Burton, MD, 13784 Highway 9, Alpharetta, GA 30004; Paul Renfroe Lewis, Jr., MS, Bioforensic Consulting, 55 Park Square Court, Ste 207, Roswell, GA 30075; and Mark C. Pozzi, MS, Sandia Safety Sciences, 2 Marietta Court, Ste A, Edgewood, NM 87015*

After attending this presentation, attendees will better understand a method for improved scientific evaluation and certification of a football helmet head-impact potential when used in common, but not currently tested, conditions of both high temperature and humidity.

Certification and evaluation of new and reconditioned football helmets should include impact performance evaluations under high temperature and high humidity soak conditions that more realistically replicate early season environmental use conditions. This presentation will impact the forensic science community by explaining how this type of evaluation can be beneficial in forensic science studies and in early phases of helmet design, and in reconditioning of used helmets, so as to assist in the proper selection of energy-absorbing padding that is more resistant to degradation of impact safety performance in the high temperature and high humidity environment.

Publicity on the dangers of concussion and severe head injuries sustained by football players subjected to repetitive head impacts has, rightfully, received much attention in recent years, in a very large part due to the diligent work by medical researchers such as Omalu and McKee; however, there is an important contributing factor related to football head injury, caused by repetitive head impacts, that has received virtually no attention thus far except for the research findings presented in this study.[1,2] That factor deals with understanding the degradation of the energy-absorbing impact characteristics of elastomeric padding materials, used in football helmets, when a player is subjected to head impacts while wearing the helmet during common situations involving both high temperature and high humidity moisture conditions, such as the conditions likely to be experienced earlier in the season when the weather tends to be both hot and humid. Current football helmet performance test certification procedures, such as the National Operating Committee on Standards for Athletic Equipment (NOCSAE) standard, do not have and never have had a test criterion for measuring helmet impact Severity Index (SI) performance when simultaneously subjected to both high temperatures and high humidity above the 90% relative humidity level.[3] The current criteria only require testing with humidity in the range of 25% to 75% levels.

In this study, two types of football helmet elastomeric Energy-Absorbing (EA) forehead impact pad designs were tested Quasi-Statically (QS) and dynamically for evaluation of load-deformation performance and head impact severity when subjected to two different environmental conditions. One environmental series of tests were run with the pads and helmet tested at ambient conditions of 23oC (72oF) and 70% humidity. A second series of tests were run with the pads and helmet subjected to a three-hour soak at 42oC (108oF) and 93% humidity. Both types of EA pads were designed in a "dual density" configuration (i.e., soft foam near the forehead and stiffer foam at the shell side), with the exception that one type of pad was encased in a Vinyl Cover (VC) and the other type had No Vinyl Cover (NVC). Both new and used pads were tested. The EA pads were 2.54cm thick and were compressed to just over 60% of full thickness in the QS tests. The QS tests showed a dramatic drop in load-carrying capability of the forehead pad when tested at the high temperature and humidity condition (see below plot). The result of this "softening" of the pad is that it becomes easier for the helmet pads to "bottom out," leading to much higher impact-load transfer to the head of the athlete.



In addition to the QS EA pad testing, dynamic full-helmet drop-impact tests were also conducted for both of the EA pad types and environmental conditions cited above. The dynamic tests used a NOCSAE head-form and vertical drop impacts into the forehead region of the helmet at impact speed levels of 5.54m/s (i.e., 12.2mph), which is the upper speed level required by the NOCSAE, and 19.05m/s (i.e., 14.2mph), which is closer to the speed of a player who can run 40 yards in just over five seconds). For the 5.54m/s impact level, the helmet with the VC forehead EA pad, when tested under the "ambient" test condition, resulted in an accelerometer peak G reading of 141 and an SI level of 645.7; however, when the helmet with the VC pad was then again tested at the same 5.54m/s impact speed, but after conditioning at the Higher Temperature and Humidity (HTH) conditions, the head form accelerometer reading indicated an increased peak G of 163 and an SI level of 786.4, resulting in a more dangerous 22% increase in the SI when tested at the 5.54m/s impact with the HTH condition versus the "ambient" test condition used for helmet NOCSAE certification. When tested with the NVC forehead EA pad at the 5.54m/s impact, the helmet results demonstrated an increase in the acceleration peak G (approximately 175) and SI measures (approximately 900), but no significant difference in the readings between the ambient and HTH. When tested at the 19.05m/s impact level, the peak G's increased, as expected, and the SI measures also increased up to, and beyond, the NOCSAE standard limit of 1,200 for both environmental conditions and both forehead pad types. It should also be noted that the HTH environmental test temperature and moisture conditions could arise from a player perspiring even if the environment levels are less than the HTH condition.

In summary, the environmentally induced changes in the load-deformation characteristics of the energy-absorbing padding shown in the curve above, and the increase in SI found in the HTH environment helmet testing, suggest that more research and testing should be conducted in the HTH area. Because of the current awareness of the "Concussion-Dangers" associated with football repeated head impact injuries brought to light by researchers such as Omalu and McKee, and because of the fact that many players of all ages play under the conditions of "High Humidity with High Temperature," at least in the earlier part of the season, the importance and need for more testing and certification of helmets under conditions of high temperature with high humidity should be of concern to all.

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Football Helmet Testing, Head Injury Severity, High Temperature Humidity

D3 Specimen Age Affects the Fracture Pattern of Immature Porcine Femurs Under Torsional Loading

Patrick E. Vaughan, BS, Michigan State University, Orthopaedic Biomechanics Laboratories, E Fee Hall, Rm 407, East Lansing, MI 48824; Feng Wei, PhD, Michigan State University, 965 Fee Road, Rm A-414B, East Lansing, MI 48824; and Roger C. Haut, PhD, Michigan State University, Orthopaedic Biomechanics, A407 E Fee Hall, East Lansing, MI 48824*

After attending this presentation, attendees will better understand the influences of bone development and rate of twist on torsional failure characteristics of porcine femurs.

This presentation will impact the forensic science community by providing additional information on the use of fracture ratio to help differentiate Accidental Trauma (AT) from Non-Accidental Trauma (NAT) under torsional loading scenarios.

In the current forensic literature, differentiation between an abusive and accidental trauma in children less than three years of age remains challenging. Currently, it is assumed that any trauma incurred by children less than three years of age is frequently NAT, and that any trauma in children less than one year is always NAT.[1] Furthermore, spiral long-bone fractures in young children are challenging cases because there are limited "ground truth" data to help forensic investigators determine the mechanisms of long-bone fracture. A recent study has proposed fracture ratio, defined as the fracture length in a lateral radiograph over the diameter of the bone, to help distinguish NAT from AT. The study has associated small fracture ratios (≈1.6) with NAT and large ratios (≈2.8) with AT.[2] Another study using an immature canine model indicates that the fracture ratio is increased for a high rate of bone twist.[3] The authors of that study assume AT occurs at a high rate of twist while NAT occurs at a low rate; however, it is currently unclear if age is a covariate of the facture ratio generated under a torsional load.

The purpose of this presentation is to: (1) present new data on torsional fracture ratios using an immature porcine model; (2) examine the effect of age on the fracture ratio; and, (3) examine the effect of age on the rate of twist sensitivity in fracture ratio.

Thirty-six immature porcine femurs aged 1-17 days were twisted to failure in a servo-hydraulic machine using a custom-built torsional loading fixture. Twenty-two specimens were twisted at a low rate ($3°s^{-1}$), among which 14 specimens were from a young age group (1-9 days) while 8 specimens were from an older group (10-17 days). These age groups were previously defined by Powell et al.[4] Fourteen additional specimens from the young age group were twisted to failure at a high rate ($90°s^{-1}$). All specimens were frozen at -20°C within 12 hours of natural death. Specimens were thawed at room temperature and tested within 48 hours. The bones were kept moist with saline solution during all preparations and experimentation. The bone ends were potted in cups with room-curing dental cement. Specimen lengths were maintained at 3.10 ± 0.44 times the smallest diameter of each bone, as measured with calipers. The fracture ratio was determined post-failure.

The study demonstrated that for bone specimens from the young group, the fracture ratio increased linearly with age for both the high and low rates of twist. For the older group of specimens twisted at a low rate, there was no significant effect of specimen age on fracture ratio. For the young group of specimens, the fracture ratio generated in the high rate of twist experiments was consistently 1.3-1.4 times that generated in the low rate of twist experiments across all ages (1-9 days). An analysis of fracture surfaces showed alternating planes of transverse and longitudinal oriented fracture to be varied with age that directly related to changes in fracture ratio.

A previous study in the biomechanical literature suggests that the helical fracture pattern often characterized after torsional loading of a long bone is due to a combination of tensile and longitudinal shear failure.[5] It has also been shown that the tensile strength of bone typically increases with age and rate of loading.[6,7] Based on the previous biomechanical literature, the results of the current study suggest that the increase in fracture ratio documented with specimen age may be due primarily to an increase in tensile strength of the bone with age and rate of twist. Additional studies will be needed to support these hypothesized changes of tensile and longitudinal shear strength with specimen age for this long bone. A better understanding of these long bone fracture characteristics may be paramount as they may have significant implications in the interpretation of fracture ratio to be used in differentiating NAT from AT in children.

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Pediatric Abuse, Fracture Pattern, Animal Model

D4 Non-Destructive Test Method for Forensic Evaluation of Motorcycle Helmet Shell Failure Mechanism and Resulting Safety Deficiency Causing Fatal Head Injury

Kenneth J. Saczalski, PhD, 1440 W Bay Avenue, Newport Beach, CA 92661; Mark N. West, BS, Environmental Research & Safety Technologists, 1440 W Bay Avenue, Newport Beach, CA 92661; Todd Saczalski, BSMET, 140 Calle Irena, Sedona, AZ 86336; Joseph L. Burton, MD, 13784 Highway 9, Alpharetta, GA 30004; Paul Renfroe Lewis, Jr., MS, Bioforensic Consulting, 55 Park Square Court, Ste 207, Roswell, GA 30075; and Mark C. Pozzi, MS, Sandia Safety Sciences, 2 Marietta Court, Ste A, Edgewood, NM 87015*

After attending this presentation, attendees will understand how to apply a method for non-destructive analysis for scientific evaluation of potential manufacturing flaws in certified motorcycle helmet shell structural failures that result in severe head injury.

This presentation will impact the forensic science community by providing a non-destructive, evidence preserving method for evaluation of potential manufacturing defects in motorcycle helmet exterior shell failures associated with severe head injuries.

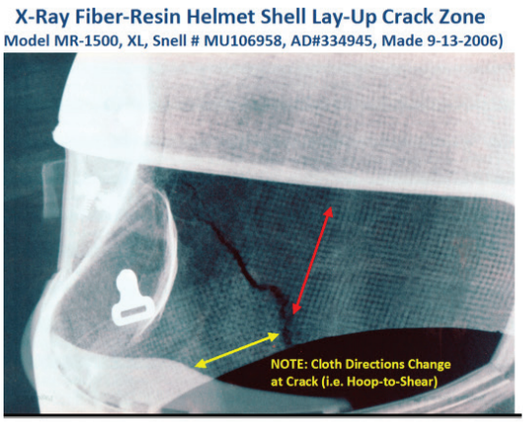
Concussion and severe head injuries sustained by motorcycle-helmeted riders using Snell and Department Of Transportation (DOT) -certified helmets are sometimes caused by manufacturing defects in the composites and plastic materials typically used in the fabrication of the critical outer protective helmet shells. The structural integrity of the outer shells is necessary to fully engage and spread the head/helmet outer surface contact impact forces over as large of a surface area and volume of the inner energy-absorbing liner as possible to minimize high-intensity focal impact forces that would otherwise transfer to localized regions of the skull/brain system and contribute to a more severe loading; however, if the shell integrity and containment load-distribution-function capability fails when the helmeted head strikes the pavement, such as when a sudden crack propagation failure in the shell occurs due to a manufacturing deficiency, the impact loads to the inner liner and head and are then concentrated on a smaller surface area, with concomitant higher focal impact that often results in severe-to-fatal load levels in the skull-brain system.

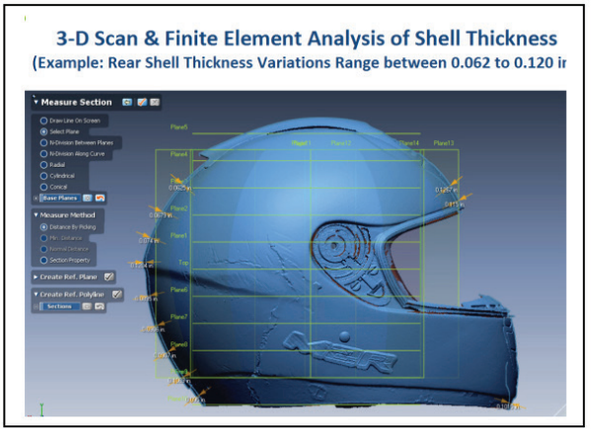
The two most common motorcycle helmet shell construction materials are the less expensive and easily fabricated injection molded thermoplastics shell, such as the polycarbonates types, and the more expensive fiber-resin laminate lay-up composite shells. Crack propagation failures in the polycarbonate helmet shell designs are typically caused by the formation of high-stress concentration impurity sites that result from mixing the more uniform and stronger virgin polycarbonate plastic material with older, used, reground plastic material obtained from the reuse of previously molded and rejected shells, which save on material-fabrication costs. With regard to the fiber-resin laminated composite shells, "resin-starved" laminates cause typical manufacturing flaws and shell stress concentration and load distribution failures. In addition, irregular laminate ply-lay-up and overlap coverage, coupled with large material thickness variations, also result in shell failure zones. The side-by-side figure below illustrates two fatal accident helmet cases in which shell fracture occurred and allowed localized loading to the skull-brain system. The left photo shows a fiber-resin composite shell failure and the right photo illustrates a polycarbonate molded shell failure.

Early research by Hodgson and Saczalski have addressed helmet performance and design issues related to the biomechanics of head-helmet loadings, and associated injury risk effects on humans, by using both analytical-numerical analysis methods and destructive impact testing of helmet systems and components; however, in order to preserve and maintain evidence in the "as-found" condition, which is needed for forensic reasons, a damaged motorcycle-accident helmet that exhibits crack failures in the outer shell structure requires the use of Non-Destructive Evaluation (NDE) techniques as well as dynamic-impact testing of exemplar helmets.[1,2] This study presents an NDE approach for identifying potential manufacturing flaws in motorcycle helmet shell structures made of both fiber-resin composites and plastic molded shells in which shell fractures resulted in severe head injuries.

The NDE method used in these helmet-failure studies involves three stages. First, the fracture sites on the outer and inner surface of the shells are carefully examined by using macro-photographic evaluation, both before any helmet disassembly is performed and after disassembly, in order to more carefully examine the damage zones of the crack region of the shell, as well as to examine the mating surface of the inner energy-absorbing liner materials. This stage of the examination documents "close-up" visual evidence of impurities in the plastic molded material shells and also reveals irregularities in fiber-resin fracture patterns in composite shell structures, often associated with "resin-starvation" or improper cure of the resin matrix chemical bonding. Second,

the helmet shell materials are examined for zones of flaws through the thickness of the material by using ultra-sound scans and X-rays to more carefully examine irregularities within the shell materials. Third, the accident shell structures are digitized with 3D scanning devices to enable accurate measurements of shell thickness variations at and around the regions of the shell fracture. Finally, if located, exemplar accident helmets are tested to evaluate several of the observed factors, including duplication of failures and consistencies of shell flaws as well as comparison with non-defective designs. The figure below illustrates an X-ray of the fiber-resin shell shown above and a "finite-element" thickness model.





The photo below shows the results from a drop-impact test run on an exemplar of the polycarbonate helmet shown on the right side in the first photo above. This test duplicated the accident helmet failure when tested according to the National Highway Traffic Safety Administration (NHTSA) helmet standard FMVSS-218. As noted above, the blending of recycled plastics with virgin materials in the molding of plastic helmet shells will likely lead to inclusions of impurities and stress concentration failure sites. The exemplar tested plastic and fiber-resin composite shell helmets allow for more detailed material defect testing in the forms of Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FTIR).

The NDE and exemplar helmet testing methodology applied in this study provides an efficient and economical means for verifying dangerous manufacturing defects in DOT- and Snell-certified helmet designs without the need for destructive testing on the actual accident helmet in evidence. Finally, it should be noted that evidence of a shell fracture does not necessarily verify a manufacturing defect unless a procedure such as that employed in this study is conducted.

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Helmet Defects, Head Injury, NDE Evaluation

D5 Fire Dynamic Simulation — Assessing Structural Damage and Suppression Potential of a Church Fire

Darren Franck, MSME, Advanced Engineering Associates, Inc, 4713 MacCorkle Avenue, SE, Charleston, WV 25304; and Harold Franck, MSEE, Advanced Engineering Associates, Inc, 4713 MacCorkle Avenue, SE, Charleston, WV 25304*

After attending this presentation, attendees will better understand the use of fire dynamic simulations to determine the extent of structural damage and suppression potential during the progression of a fire.

This presentation will impact the forensic science community by demonstrating analytical tools to determine culpability in the protection of a historical structure.

This case study details an investigation of a fire at a historical church in Provo, UT. The church's construction dated to the 1880s and included much of the original framing. The attic contained a plywood deck that was installed in the 1980s, at which time smoke detectors were installed within this space. In late 2010, the church was holding an annual Christmas play. Alterations to the lighting system were made temporarily for the play. The changes included removal of the existing light fixtures in order to descend a decorative lighting truss through the ceiling. The contractor performing this work placed one of the fixtures on the plywood deck; however, he failed to turn off the light, which eventually led to a smoldering fire. Although some witnesses smelled smoke during the play, no one had been in the attic since the lighting system was placed in operation. Some time after the end of the play, burning embers breached the perimeter of the fixture and developed into a full-scale fire. This fire progressed through the attic and eventually consumed much of the roof, which led to a partial collapse of the structure.

The cause of the fire, while accidental, resulted in action against the lighting contractor; however, pertinent issues of suppression potential and a failure to recognize the fire were raised. The fire was not discovered until several hours after the end of the play. Past false alarms in the smoke detection system confused the security detail during the initial alarms. This confusion resulted in a 90-minute delay in responding to the fire, at which time the fire began to breach through the attic floor and into the ceiling of the church. In order to assist in a determination of the effects of this time delay on the condition of the structure and suppression potential, representatives with the church commissioned a fire dynamics simulation of this event.

The investigation began by reviewing the findings of the fire marshal, who was intimately involved in fighting the fire and in determining its cause. Modeling the fire required detailed knowledge of the dimensions of the church and materials used in the construction, especially within the attic. The shell of the structure was still in place at the time of the site visit; however, considerable efforts were underway to salvage the original brick walls and to expand the depth of the foundation. These efforts, as well as the extensive gutting of the church following the fire, limited the information available for use in the model. Information based on the as-built diagrams, plans devised during past remodeling, and the photographic record assisted in reconstructing the roof frame and the contents of the attic.

Fire Dynamic Simulation (FDS) and Smokeview software was used to perform the analysis. This software was developed by the National Institute of Standards (NIST) and has been widely used in both design of fire safety equipment and the reconstruction of fire events. FDS is a computational fluid dynamics model of fire-driven fluid flow. Smokeview is a visualization program for the results of the simulation. Input variables and output files are written into Fortran code and solved within the FDS program. Computing these results for a large-scale fire over a long period of time proved challenging for both the analysts and hardware.

Modeling the fire involved setting global boundaries, defining cell sizes, assigning materials (i.e., wood and metal) to the solid items within the attic, and setting the geometry for these items. Ventilation at the ridge and gable ends was also included in the model. In order to limit computational time to periods of days rather than weeks, the cell sizes were selected toward the maximum limit recommended by the program developers. The large cell sizes limited assessment of the progression of the fire within the interstitial spaces of the ceiling frame. Additionally, the program requires sloped and curved objects to be modeled in Cartesian coordinates, which creates vortices at the resultant corners that limited the ability to complete some simulations. This problem was alleviated by a modification to the software code; however, these changes limited the amount of information gleaned by the analysis.

The results of the analysis were consistent with the time frame developed by the original fire investigators. Output data included burning rates, gauge heat flux, soot mass fraction, as well as surface and air temperatures. Soot levels indicated that the smoke detectors would have been activated within a few minutes after the smoldering fire breached the perimeter of the light fixture shield. Testing performed by the fire marshal indicated that the smoldering fire would develop into open flames once air was introduced into the fuel mixture. The duration of this test is consistent with witness observations during the play and the timing of the smoke alarms. Thus, the delay in responding to the fire was no greater than 90 minutes.

Still, this represents a considerable time for open flames to spread throughout a large, open attic. The fact that the roof frame was composed of wood that had dried for more than a century in a desert environment likely increased the rate of consumption. The results of the analysis confirmed the decision by the fire chief to avoid entering the attic after the fire was ultimately discovered. The extent of damage to the framing members and temperatures within the attic precluded an interior attack. The limitation of the suppression efforts to a defensive approach prevented any successful salvaging of the structural elements. The actions of the security company were assessed, namely their failure to recognize the signs of a fire and respond to the alarms;

however, a few conditions that were out of the security company's control factored into these failures.

Fluid, Soot, Burning Rate

D6 Application of Reverse Engineering in Forensic Investigation

Wego Wang, SciD, 205 Bristol Road, Wellesley, MA 02481*

After attending this presentation, attendees will be able to: (1) apply appropriate reverse engineering methodology to their forensic analyses; (2) analyze the root cause and explain the circumstance of an accident by applying the principles of reverse engineering; and, (3) collect the relevant evidence as a subject expert witness in a forensic investigation based on reverse engineering practices.

This presentation will impact the forensic science community by assisting attendees to better understand the scientific methodology and engineering analysis in reverse engineering, their feasibility and applicability in forensic investigations, and the roles they have played in many recent successful cases. This presentation will discuss how to use everyday cameras and apply the principles of photogrammetry to create drawings and 3D models of objects pertinent to forensic investigations, such as the details of an involved crime site or building layout.

Most forensic investigations begin with the "apparent" end result, such as a burned-down house, collapsed bridge, damaged machinery, or wrecked vehicle. From this starting point, the forensic investigator collects data and gathers evidence to "reverse engineer" what has happened and how it happened. The application of reverse engineering to forensic investigation can help resolve the questions pertinent to who, what, where, when, why, and how from an engineering perspective in particular.

The availability of modern technologies in reverse engineering plays a pivotal role in many successful forensic investigations. This presentation will first briefly discuss the principles of reverse engineering, then focus on its applications in various real-life forensic investigations relative to transportation accidents, natural disasters, and personal injury. In these case studies, both the merits and limitations of reverse engineering will be discussed, as are the potential impacts of reverse engineering in future forensic investigations during the next decade.

Determination of the residual chemicals in fires caused by chemical explosions is essential. This presentation will discuss the advanced reverse engineering methods used for elemental material identification. For example, Inductive Coupled Plasma/ Optical Emission Spectroscopy (ICP/OES) is widely utilized for the determination of major component concentrations; however, it is subject to severe restrictions in the analysis of trace elements. For trace elements detection, glow discharge mass spectrometry is a more appropriate tool. On the other hand, for determination of included gases in a solid sample (e.g., carbon, sulfur, nitrogen, oxygen, and hydrogen) interstitial gas analysis is a more appropriate tool. This presentation will also discuss other readily available reverse engineering methods for chemical detection such as X-ray fluorescence analysis and techniques for analyzing coating materials.

During a forensic investigation, the photographer takes images from the 3D world and projects then onto a flat 2D image plane, but depth of information is lost. Photogrammetry reverse engineers this process. By knowing some information about the camera that took the photographs and by having a few more photos of the same object from a different perspective, some lost 3D information can be regained. The entire sets of 3D measurement data at accident or crime scenes could be captured by simply making the photos "photogrammetry friendly." A few skillful photos can make photogrammetric analysis easy.

Reverse Engineering, Forensic Investigastion, Chemical Analysis

D7 Evaluating the Structural Failure of Wood Bowstring Trusses Under Heavy Snow Loading

Daniel M. Honig, PE, Structures Consulting Engineers, PO Box 125, Swarthmore, PA 19081*

After attending this presentation, attendees will understand the factors which contributed to the cracking, structural failure, and/or partial collapse of several bowstring truss elements within a roof framing system, as well as the means and methods through which such a condition should be corrected to constitute safe occupancy of the building.

This presentation will impact the forensic science community by illustrating how uniform snow loads can negatively impact the structural integrity of roof framing members, particularly bowstring trusses, which are often subject to potential overstress under such conditions. This presentation also highlights the importance of thorough structural inspection and proper remediation methods, which can prevent buildings from experiencing complete structural failure of the roofing system.

During winter months, uniform or unbalanced roof snow loads can create stresses on the framing members of a roofing system; the weight of snow adds to the dead load of the system itself, supported by the members. This can lead to significant deflection and, over time, potential structural failure of these framing members. These conditions become more prone to occur as structural wood ages and experiences normal loading.

In this instance, the conditions experienced during the 2010/2011 winter season brought unusually cold temperatures for the Pennsylvania region, in addition to an increase in snowfall compared to preceding years. The unusual quantity of snow, in conjunction with lowered temperatures, led to the notable downward deflection and significant cracking in 6 of the 15 wood bowstring truss roof supports. These conditions were not localized to any particular roof area, rather they were widespread throughout various locations in the structure, furthering the evidence that these problems were caused by excessive snow loading across the roofing surface.

To properly remediate the damage to the trusses, and ensure the structural stability and safe occupancy of the building, computer analysis was performed using Rapid Interactive Structural Analysis for Two-Dimensional Planar Structures (RISA-2D) in order to evaluate the effects of varying live or dead loading conditions on a typical bowstring truss. These analyses found that under certain loading conditions, the bottom chords and web members of the bowstring trusses would experience twice the design stress for uniform snow loads, which could result in fracture, splitting, and possible structural failure of the trusses. Additionally, the presence of skylights within the building's roofing system were found to not only add to the dead load supported by select local trusses, but increase potential snow loading as a result of surrounding snow drift accumulation.

A structural remediation plan was detailed to properly reinforce and repair the bowstring trusses as needed, and thereby prepare the members to adequately support any snow loading during the impending winter season. It was recommended that tension reinforcements be added along the bottom chord of all members, in addition to the installation of reinforcement members along the lengths of the diagonal web members, which were determined to be insufficiently sized. The truss conditions also required the installation or replacement of bridging and diagonal bracing. Plans for adequate temporary shoring to prevent more significant damage or structural failure were also outlined, given that the initially installed temporary shoring was structurally inadequate to provide the support needed to prevent the roof framing system from experiencing failure during the upcoming winter season.

Bowstring, Failure, Snow

D8 Comparison of Measurement Error Between 3D Laser Scanning, Total Station Survey, and Photogrammetry Using PhotoModeler®

Shannon Wilson, J2 Engineering, 5234 E Pine Avenue, Fresno, CA 93727; James E. Flynn, BS, J2 Engineering, Inc, 5234 E Pine Avenue, Fresno, CA 93727-2109; Stephen Harper, BS, J2 Engineering, 5234 E, Fresno, CA 93727; and Jace Priester, BS, Three Space Imaging, 4690 E Peralta Way, Fresno, CA 93703*

After attending this presentation, attendees will be able to assign a confidence level to the method of measurement chosen for their specific purpose.

This study will impact the forensic science community by providing expert witnesses with information regarding the potential levels of error when dimensioning an object using a 3D laser scanner, a total survey station, or PhotoModeler® photogrammetry software.

The devices and software used were a FARO® Focus^{3D} laser scanner with SCENE software, a Trimble® 5000 series total station survey system, and photogrammetry using PhotoModeler® 6 to analyze photos and take measurements. All three methods were compared to known dimensions as determined with a steel tape. Three separate measurements were taken at various points on a tool cabinet. The measurements were taken at the points located at the edge of the wooden top, along the back supporting column, and from a diagonal located along the face of the base (see photographs below).

The FARO® scanner was used at high and standard definitions. The settings at high definition were set to ¼ resolution, which is a point density of .221 inch spacing at 30 feet, and 6x quality, which measures the same point six times and averages the distance. The settings at standard definition were set to ¼ resolution, and 4x quality. Scanning at standard definition required only one-third of the time needed to complete a high-definition scan. Four scans were taken at each setting and were registered by using SCENE software. Each measurement was taken three times and averaged for comparison to the steel tape. The Trimble® total station survey system used direct reflection for point gathering. The points were downloaded and labeled in the Trimble® office application and exported as a .dxf file. The .dxf file was then opened in AutoCAD® and measurements were taken by measuring the 3D length between points. Photos of the cabinet were taken and imported into PhotoModeler®, which was used to construct a 3D model of the cabinet. Measurements between the indicated points were obtained using the PhotoModeler® measuring tool. To obtain the diagonal measurement, an additional line was drawn and measured.

Error was determined by determining the difference between the digital measurement and the steel tape measurement for each point. The calculated percent errors were averaged for comparison. The average percent error for the high definition scans, standard definition scans, total survey station, and PhotoModeler® measurements when compared to the steel tape are 0.18%, 0.27%, 0.38%, and 0.36%, respectively. The maximum percent error for the high definition scans, standard definition scans, total survey station, and PhotoModeler® measurements when compared to the steel tape were 0.22%, 0.33% 0.49%, and 0.63% respectively (see tables below).

High Definition Scanner vs. Steel Tape

	Steel Tape	Scanner	% Error	Error in In.
1	59.375	59.256	0.20042	0.119
2	67.75	67.652	0.14465	0.098
3	65	64.856	0.22154	0.144
AVE			0.18887	0.120333333

*Presenting Author

Standard Definition Scanner vs. Steel Tape

	Steel Tape	Scanner	% Error	Error in In.
1	59.375	59.176	0.33516	0.199
2	67.75	67.6	0.2214	0.15
3	65	64.84	0.24615	0.16
AVE			0.26757	0.169666667

Photogrammetry vs. Steel Tape

	Steel Tape	Photo	% Error	Error in In.
1	59.375	59.496	0.20379	0.121
2	67.75	68.085	0.49446	0.335
3	65	65.278	0.42769	0.278
AVE			0.37532	0.244666667

Survey Station vs. Steel Tape

	Steel Tape	Survey	% Error	Error in In.
1	59.375	59.0016	0.62888	0.3734
2	67.75	67.7964	0.06849	0.0464
3	65	65.2416	0.37169	0.2416
AVE			0.35635	0.220466667

Digital Dimensioning, 3D Scanning, Error

*Presenting Author

D9 Natural Language Engineering for Multilingual Forensic Author Identification

Carole E. Chaski, PhD, ALIAS Technology, LLC, Institute for Linguistic Evidence, 25100 Trinity Drive, Georgetown, DE 19947; Nan Decker, PhD, ALIAS Technology LLC, 25100 Trinity Drive, Georgetown, DE 19947; Ali M. Alshehri, MA, University of Buffalo, Dept of Linguistics, Buffalo, NY 14260; Seung-Man Kang, PhD, Chungbuk National University, International Services Center, Cheongju, SOUTH KOREA; and Angela Almela, PhD, Universidad Catolica de Murcia, Campus de Los Jerónimos, 135, 30107 Guadalupe, Murcia, SPAIN*

After attending this presentation, attendees will be aware of the challenges and opportunities for English, Arabic, Korean, and Spanish forensic linguistics, including natural language engineering toolkits that are being developed for specific forensic linguistic tasks.

This presentation will impact the forensic science community by demonstrating that forensic linguistic tools are becoming available for English, Arabic, Korean, and Spanish, which can aid in both international and national investigations.

The role of Natural Language Engineering (NLE) is increasingly recognized as necessary in forensic linguistics. The most successful approaches to authorship identification of small texts are based on syntactic patterns. Generally, the texts are tagged and parsed by parsers — a fundamental tool in NLE — with the subsequent syntactic patterns categorized, counted, and the output analyzed statistically.[1-6] The NLE approach faces two challenges that must be overcome for accurate forensic author identification: messy data and multilingual data.

First, most taggers and parsers have been built on edited text, such as novels or news writing, which abide by predictable orthography and syntax. But most forensic linguistic texts are unedited. Texts of unedited language are full of ungrammaticality, non-standard spelling, and variable punctuation. Such naturalistic texts are very difficult for part-of-speech taggers and parsers. The problem of parsing ill-formed input or ungrammatical sentences was first discussed more than 30 years ago, and it remains an incompletely solved problem.[7-12] The difficulty of tagging and parsing ill-formed data has important consequences within the forensic context. In the forensic application, any error during a procedure can introduce reasonable doubt that the entire procedure's result is flawed. Therefore, forensic author Identification (ID) methods that rely on parsing using standard parsers without any concern for how well or poorly the parser handles ill-formed input may not be as accurate as reported since the parses upon which subsequent analysis relies cannot be trusted as accurate.[13] Even clean data from legal argumentation cannot be perfectly tagged/parsed by commercial tagging systems; in a recent test of nearly 100,000 words from legal publications, nearly 6,000 words (or 16%) required correction of the tags. Therefore, if the forensic linguistic method handles the data processing either through manual error checking or automatic error assessment, then the total procedure of automated authorship ID safely handles the ill-formed input of truly natural language typical of forensic linguistic data.

Second, most taggers and parsers have been built for English, with other world languages having fewer tools available for NLE. Most of the work in forensic linguistics has been concentrated in the English language, but this Anglocentric focus is untenable in the current geopolitical climate.[14-16] Thus, work has begun on developing a multilingual foundation for forensic linguistics using NLE.[17] This presentation reports on a series of experiments that test the accuracy of multilingual parsers on messy data in English, Arabic, Spanish, and Korean. Data was gathered from: (1) the internet using blogs and social media; and, (2) experimental protocols using known subjects who are native speakers of English, Arabic, Spanish, and Korean.[18] The documents are run through taggers in the Text Analysis Toolkit Toward Linguistic Evidence Research (TATTLER) system. Linguists with graduate degrees manually check the tags, correcting any that are erroneous. TATTLER automatically calculates the errors per sentence, document, and language data.

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Natural Language Engineering, Forensic Linguistics, Multilingual

D10 Analysis of Citrate Distribution in Bone for the Estimation of Postmortem Interval

Matthew Pysh, 325 Orange Court, Clemson, SC 29631; Katherine E. Weisensee, PhD, Clemson University, Dept of Sociology & Anthropology, 132 Brackett Hall, Clemson, SC 29634; Mark A. Schlautman, PhD, Clemson University Environmental Engineering, Geological Sciences Coordinator Office, 441B Brackett Hall, Clemson, SC 29634; and Melinda Harman, PhD, Clemson University, Dept of Bioengineering, 105 Sikes Avenue, Clemson, SC 29634*

After attending this presentation, attendees will better understand the distribution of citrate along porcine rib bones as well as variance between individuals.

This presentation will impact the forensic science community by providing results for an area of research that is lacking in literature. This presentation will add to research previously performed to evaluate the potential of citrate concentration as a means to estimate postmortem intervals by broadening the understanding of the distribution of citrate within porcine rib bone samples to better determine sampling requirements for the use of this method in forensic investigations. Furthermore, this presentation will provide and outline the methods needed to evaluate citrate concentrations using High-Performance Liquid Chromatography (HPLC).

Estimation of the Postmortem Interval (PMI) can be critical in cases involving decomposed human remains. Current techniques of PMI estimation are based on soft tissue decomposition, but these techniques are generally useful only in the active stages of decomposition. Alternative methods using skeletal remains have been investigated as a means to circumvent the limitations of soft tissue decomposition techniques. One of the most promising and recent techniques of PMI estimation of skeletal remains has sought to utilize the degradation of citrate within bone, which accounts for nearly 80%-90% of all citrate in the body.[1-5] The earliest studies using this method suggested the ability to use citrate content of bone to estimate PMI of up to approximately 100 years with a 1% error in the PMI determination.[5] Subsequent studies used the citrate method to compare estimated PMIs with known values to evaluate the success of this technique. Overall, it was determined that the method did not provide consistent results. An analysis of these studies demonstrated the need for a better technique for determining citrate concentrations and the need for a better understanding of citrate distribution within bone, as well as citrate variations among bones from different individuals.

In this experiment, two fresh racks of porcine ribs were acquired and subsequently frozen until use. The length of each rib was measured and subsequently divided into three equal-length sections based on the total length of the bone. The midpoints of each of the three sections (dorsal, ventral, and central) were identified and marked on the bone. Sections of bone were then cut to obtain one 2mm-4mm bone specimen. Immediately after sectioning, each specimen was processed through a bone-processing protocol and prepared for HPLC analysis. Citrate concentrations, in wt% from the cortical bone in each of the specimens, were recorded and compared to gain an understanding of the citrate distribution along individual bones, as well as the citrate variation across individuals.

A preliminary study using the method described above has suggested varying citrate concentrations in different portions of bone. Initial statistical testing has indicated a significant ($p<0.05$) difference between the ventral and dorsal areas of the bone. These preliminary results examined variations in a single individual; however, additional analyses are needed to determine whether the difference presently observed is consistent across individuals. Further analysis will determine how age and other factors influence citrate concentration in different bones and portions of bone.

In conclusion, this study seeks to provide a better understanding of citrate variation along bone to better evaluate the potential of a citrate-based method for determining the PMI. Early data has suggested that a large variation in citrate concentrations can be found depending on the location of sampling. Moving forward, this research seeks to gain more confidence through testing of different individuals. Overall, this study should help to provide more information on potential errors associated with the use of a citrate-based method for PMI estimation.

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Postmortem Interval, Citrate, HPLC

D11 Crime Scene Imaging Using a Highly Affordable, User-Friendly, Portable, Open-Source 3D Imaging System

Nikolaj Kjaer Nielsen, Aarhus Univerisity Dept of Engineering, Skovbakkevej 21 lej 18, Brabrand 8220, DENMARK; Kim Juul Henriksen, BS, Aarhus School of Engieneering, Finlandsgade 22, Aarhus N 8200, DENMARK; Samuel Alber Trysoee, PhD, Aarhus School of Engineering, Finlandsgade 22, Aarhus N 8200, DENMARK; Henrik Pedersen, PhD, Dept of Engineering, Aarhus University, Finlandsgade 22, Aarhus N 8200, DENMARK; and Iana Lesnikova, MD, PhD, Havkaertoften 14, Tilst 8381, DENMARK*

After attending this presentation, attendees will be familiar with a highly affordable, open-source, fast-acting, portable imaging system able to perform a 3D scan of the body and surrounding areas during the crime scene investigation. The output is a colorized, dimensionally stable, and interactive 3D image, with a resolution of less than $1in^3$, thereby allowing clear visualization of small items, such as cigarettes or pencils.

This presentation will impact the forensic science community by creating awareness of new approaches in crime scene imaging. 3D crime scene imaging is thought to be a promising supplement to 2D photography or video and thereby provide an additional source of evidence. The method takes advantage of the modern 3D imaging technique, allowing rapid, accurate, and dimensionally stable 3D snapshots of the crime scenes. The presented solution is license free, independent of network or cell phone connection, comparatively inexpensive, and portable.

The examination of homicide scenes and other suspicious or obscure cases of death prior to body removal is an important part of the forensic pathologist's work. At the scene of death, the forensic pathologist records the body position, local environment, clothing, relation of the body to nearby objects, and condition of the body. According to current trends, forensic pathologists rarely tend to be involved in crime scene investigations, and thus important information that can only be acquired at the crime scene may be lost. Therefore, good documentation of the crime scene area will increase the quality of the forensic examination and provide more powerful conclusions. 3D imaging is believed to be a promising tool to positively expand this documentation.

The proposed method uses existing mass-produced technologies targeted at the gaming and entertainment industry. By using these technologies, it is possible to reduce the cost of a 3D scanning system by an order of magnitude. Generally, the concept is based upon projected, infrared, optical-scanning category, 3D mapping equipment produced for gaming systems. This specific type of technology has been used in many research projects regarding motion, gesture tracking, 3D mapping, and volumetric reconstructions. Therefore, this category of equipment has a fairly extensive documentation and provides a good research base, especially within compatible software and open software libraries for full utilization of the hardware capabilities.

To gather and process image information, an open-source software platform was developed. It constructs the 3D image and presents it in a manner which requires a minimum of time and technical knowledge. Figure 1 illustrates the visual results of the system, in its early development stages.

Figure 1: 3D model of a living test subject

The system is constructed in a portable manner by utilizing off-the-shelf hardware and open-source software in such a manner that construction of the system can be accomplished by a person with a normal understanding of the technology.

The study and development of the system was conducted in cooperation with the Institute of Forensic Medicine, University of Aarhus, and was tested in real-life scenarios.

Forensic Imaging, 3D Scanning, Open Source

D12 Is the Gatekeeper Concept Failing the Justice System? Is There a Viable Alternative?

John Nixon, CEng, MBA, ARC, PO Box 66, Bippus, IN 46713*

After attending this presentation, attendees will better appreciate what can go wrong when poorly vetted experts are ineffectively challenged by opposing attorneys, and then slip by judicial gatekeepers who may be unable to detect their flaws and are unwilling to reject them as witnesses. Options for improvement will be discussed.

This presentation will impact the forensic science community by alerting them to the inadequacy of current expert vetting procedures with particular regard to the role of the judge as gatekeeper. The forensic science community will be motivated to consider alternatives to the current system.

The featured case study focuses on a criminal murder case — an ongoing case that has been litigated for 21 years, during which time the defendant has been convicted of murder in two trials. Thirteen years into the litigation, prosecutors unveiled a new expert following revelations that one of their original experts had provided critical trial testimony that was diametrically opposed to information recorded in his previously undisclosed bench notes. Additionally, the original expert, with 37 years on the job, did not have the two degrees that were claimed on his curriculum vitae, and an academic transcript that he had provided was a forgery. The new expert subsequently gave some questionable testimony in a post-conviction hearing for a new trial, and appeared to be trying to justify the work of the discredited expert rather than embarking on an independent fact-finding quest.

In this case, several judges and several teams of attorneys failed to notice that an affable and confident expert was, in fact, a dishonest charlatan. That expert had testified in thousands of trials over the course of his 37-year career so, presumably, hundreds of judges and attorneys had failed to recognize him for what he was. A modicum of background research by any of the countless attorneys and judges who had allowed this witness to testify would have ended the charade. So why did not one of them do it in all that time? Or did they try and fail?

The case raises a number of issues relating to the prevailing system of having judges act as 'gatekeepers' with regard to expert qualification and testimony. Is it unrealistic for judges to rely solely upon the due diligence of opposing attorneys to provide evidence to dispute the integrity and expertise of a proposed witness? Are opposing attorneys capable of exposing bogus experts?

In a world of rapidly expanding scientific disciplines, perhaps using the courts as gatekeepers can be marginal at best and disastrous at worst. Is there a better way to ensure reliable, competent, and honest expert testimony? Should it be left to the diligence of legal teams to vet opposing experts thoroughly and engage their own expert to present an opposing view — thereby allowing the jury to weigh credibility? Perhaps the court would be well served by a third independent expert to provide expert analysis of the work of the experts of both sides — but do such independent experts exist, and how could their independence be assured?

Expert Verification, Expert Testimony, Gatekeeper

D13 The Federal Bureau of Investigation's (FBI's) Misrepresentation of Hair Evidence: History, Response, and Remedy

Peter D. Barnett, BS, Forensic Analytical Sciences, 3777 Depot Road, Ste 403, Hayward, CA 94545*

After attending this presentation, attendees will understand a brief history of the problems with the use of hair as a means of personal identification. The FBI's response to a determination that such evidence has been misrepresented in court by FBI examiners and a possible solution to such misrepresentation of evidence generally will be proposed.

This presentation will impact the forensic science community by explaining how unreliable scientific evidence is introduced at trial and how methods to prevent such introductions can be devised. Frequently, the problem lies in the content of the testimony, not the underlying science. Restricting expert witness testimony to the opinions and conclusions stated in a written report prepared for review prior to the trial could prevent such "blind siding" testimony at trial.

Hair is a potentially valuable form of evidence: It is universally present in essentially all mammalian species. Shed hairs are physically robust and not easily degraded. Casual observation reveals a great deal of variation of hair from one individual to another. Hair is a complex material with a great variation in the characteristics of features that can be discerned by a microscopic examination. Forensic scientists hoped to exploit the complex variability of these characteristics to determine that a hair recovered from a crime scene was from a particular individual. They recognized, and stated in reports, that human hair is not an absolute means for human identification, or words to that effect.[1]

With the advent of the ability to obtain genetic information from analysis of hair samples, it became apparent that there were instances in which a hair associated with a particular individual was not actually from that individual. As cases involving hair evidence were reviewed by various post-conviction organizations such as the Innocence Project, it was found not only that many reported hair associations were wrong, but the testimony presented to the juries vastly overstated the value of the evidence.[2] Misrepresentation of the value of hair evidence, by the FBI laboratory examiners and by other hair examiners, at least some of whom had been trained in the FBI laboratories, continued for 20 years.[3]

Potential problems in the FBI examination of hair evidence were described in 1981.[4] When testimony of FBI hair examiners came to the attention of the criminalistics community, it was widely agreed that such testimony had no basis in fact and was severely misleading. Finally, in 2015, following a growing recognition of errors in the association of a hair with a particular individual, and the frequent gross representation of the value of hair evidence by FBI examiners, the FBI instituted an investigation by the Inspector General.[5]

The reasons that FBI, and other, examiners reached and expressed such erroneous conclusions are many and varied. That this evidence and accompanying testimony came to be presented in so many trials underscores the ineffectiveness of the legal system to deal with scientifically false or unreliable expert testimony.

Attempts to ensure that scientifically reliable testimony is presented in trials have relied mostly on the ability of judges to determine that the basic technology involved is scientifically valid.[6] The admission of wrong, deceptive, or misleading opinions about hair evidence proves the deficiency in this approach. The problem is generally not the underlying technology; it is rather the overreaching expert testimony, aided and abetted by aggressive advocates. The courts explicitly permit this type of testimony.[7] Often, the testimony is unanticipated by opposing counsel and therefore goes to the jury relatively unchallenged. The oversight of judges in this context is ineffective.

Judges may not be able to evaluate the validity of the science behind the opinion offered by a scientist but may be able to determine whether the opinions and conclusions expressed by a scientist in an oral presentation are the same as the opinions and conclusions expressed by that scientist in a written report prepared in advance of the trial and made available for review by other scientists.

Lawyers facing the use of scientific evidence in court should have the ability, responsibility, and funding to obtain the assistance of knowledgeable scientists to review the work of scientists retained by other parties. All scientific evidence in a case should be reported in scientifically acceptable, written reports. Reports should be prepared in a time and manner to allow other scientists to review and respond to those reports. Reports should be available to the jury and judges. Oral testimony from scientific witnesses, if allowed, should be strictly limited to those opinions and conclusions expressed explicitly in the report prepared by the scientist.

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Testimony, Scientific, Hair

D14 Quantification of Forces Generated by Volunteers in Stabbing Trials

Gary Nolan, BS, East Midlands Forensic Patholo, Level 3, Robert Kilpatrick Cli, Leicester Royal Infirmary, Leicester LE1 7LX, UNITED KINGDOM; Sarah V. Hainsworth, PhD, University of Leicester, Dept of Engineering, Leicester LE1 7RH, UNITED KINGDOM; and Guy N. Rutty, MD, University of Leicester, Forensic Pathology Unit, Robert Kilpatrick Bldg, Leicester LE2 7LX, UNITED KINGDOM*

After attending this presentation, attendees will understand the different levels of force that can be generated by volunteers stabbing a dynamometer. Attendees will appreciate the differing levels of force generated by men and women and will understand how these stabbing forces relate to the force necessary to penetrate the skin.

This presentation will impact the forensic science community by showing how engineering measurements of force can be used by forensic pathologists to help interpret the levels of force required in stabbing attacks.

Stabbing is the most common way of committing murder in the United Kingdom. The Crime Survey for England and Wales released in July 2015 revealed that the police recorded 26,370 offenses involving a knife or sharp instrument, which was a 2% increase for the year ending March 2015 over the previous year. Most of the increase was in the category of "assault with injury and assault with intent to cause serious harm," which was up 13%, but this was partially offset by a 14% decrease in robbery offenses using knives or sharp weapons. Knives and sharp weapons are therefore a major crime problem in the United Kingdom and other countries where guns are not readily available.

Some of the key forensic questions that are relevant to stabbing are how much force is required to create a particular stab wound and, in particular, how much force does a person stabbing with a particular instrument generate and how does this relate to everyday actions that can be communicated to juries.

In order to address these questions, this presentation reports on the development of a dynamometer for the measurement of force during stabbing with various implements into pork skin (a human skin analogue) or a skin simulant system. The dynamometer consists of two aluminum plates that are instrumented by force cells. The peak force generated by male and female volunteers was recorded for a range of scenarios using different implements and with different stabbing methods.

To analyze the data collected from the dynamometer, mixed effects linear regression was used. Since the experiment involved collecting multiple independent and continuous variables, mixed effects linear regression allows statistical analyses to be performed to understand whether the different variables had a statistically significant influence on the recorded results. Mixed effects models allow the use of both fixed and random effects in which the variables are inter-dependent rather than independent. This means that simple linear regression methods are inappropriate.

The results of these investigations demonstrated that men typically generate twice as much force as women when stabbing and that in the majority of cases, people generate higher forces with their dominant hand. The force generated in stabbing events is much greater than that required for penetration by a knife and, in almost all cases, volunteers were able to stab and penetrate both pork and the skin simulant system with a small amount of force.

In conclusion, the key issue in deciding whether a weapon creates a sharp force injury (stab wound) during a stabbing attack is whether the threshold force for penetration with that particular implement is met; therefore, it is critical to understand whether or not the tip of the weapon permits penetration. If the weapon penetration force is less than the force generated by stabbing, the victim's skin and underlying fat and muscle will always be penetrated.

Figure 1: A volunteer performing a stab into the dynamometer. Force data is taken from three load cells and the system was calibrated with known applied forces to ensure the peak forces that were recorded were accurate.

Stab, Force, Penetration

D15 Identification of Building Insulation and Soundproofing Products by Light and Electron Microscopy

Richard S. Brown, MS, MVA Scientific Consultants, 3300 Breckinridge Boulevard, Ste 400, Duluth, GA 30096-893*

After attending this presentation, attendees will better understand how Polarized Light Microscopy (PLM), Scanning Electron Microscopy/Energy Dispersive X-ray Spectrometry (SEM/EDS), and Analytical Electron Microscopy (AEM) were used to determine the composition of insulating and soundproofing materials.

This presentation will impact the forensic science community by providing an analysis procedure that was developed over several years and used to determine the composition of insulating and soundproofing materials so they could be compared to the manufacturers' formulas for the purpose of cost-recovery litigation to reimburse the building owner for the cost of abatement.

When the Environmental Protection Agency (EPA) was tasked in the late 1980s with the implementation regarding the inspection of schools for Asbestos-Containing Materials (ACM) and the decision to remove materials that were friable, litigation resulted in the acquisition of formulas used by companies identified as producing ACM. The analysis methodology developed, once the formulas were decoded and the array of terminology used for raw materials by different companies was understood, required the microscopical characterization of the ingredients utilizing PLM, SEM/EDS, and AEM (Transmission Electron Microscopy/Energy Dispersive X-Ray Spectrometry (TEM/EDS) with selected area diffraction) on each sample.[1] By analyzing the sample using this combination of microscopical techniques, a cross-check was provided during the analysis ensuring that all of the ingredients were accurately characterized. For example, if PLM detected gypsum, vermiculite, and montmorillonite clay, then SEM/EDS and AEM would be expected to find the same ingredients. Other analytical techniques such as X-Ray Diffraction (XRD), Gas Chromatography (GC), loss on ignition, and acid-soluble weight percents were employed when the sample matrix required additional work.

Once a building survey was completed, samples were examined for the presence of asbestos using the regulatory definitions of the fibrous asbestos minerals amosite, chrysotile, tremolite, actinolite, anthophyllite, and crocidolite. Samples were then submitted to the laboratory to determine the composition of the material for comparison to formulas provided by the courts. Samples were photographed "as received" to document number and layer sequence. Multi-layered samples were treated as multiple samples requiring a complete analysis of each layer. PLM analysis was performed using slide mounts prepared to obtain visual estimates of the materials comprising each sample. Sub-samples were obtained and distributed for acid-soluble weight percent, SEM/EDS, and AEM analysis. Samples containing low amounts of acid-insoluble materials required the reanalysis of the acid-insoluble material collected on the filter during the acid digestion procedure using 10% v/v hydrochloric acid. Mineral wools could be removed from most samples by heating the 10% HCl solution and dissolving the mineral wool.

Although the types of asbestos fibers present were important, the materials were sorted based on their major, minor, and trace components. Many products could be differentiated based on their determined composition when compared to the formulas provided. Some products were very similar among manufacturers and could not be differentiated by microscopical techniques alone. Major components such as gypsum, Portland cement, glass fibers (including mineral wools), clays, and diatoms (including synthetic materials produced from diatoms) were identified using the combination of microscopical techniques described.[2,3] AEM was especially useful for the asbestos identification, clay identification, and other crystalline minerals used in the various formulations.

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Microscopy, Dust, Insulation

D16 Forensic Microscopy in a Case of Asbestos-Containing Cigarettes

James Millette, PhD, Millette Technical Consultants, 220 Cricket Walk, SW, Lilburn, GA 30047*

After attending this presentation, attendees will better understand how microscopy can be used to study evidence in an unusual civil asbestos case involving vintage (more than 50 years old) cigarettes made with filters containing crocidolite asbestos (Micronite® filter Kent® cigarettes).

This presentation will impact the forensic science community by providing information about handling and analyzing vintage samples to confirm their composition and to determine whether exposures to asbestos could have occurred in the 1950s when the Micronite® filter Kent® cigarettes were commercially available.

During the period of approximately March 1952 through May 1956, the Micronite® filter in Kent® cigarettes used crocidolite asbestos as part of the filtering agent. There was no barrier or secondary filter between the end of this filter and the customer's mouth. It has been estimated that approximately 585 million packs (more than 11 billion cigarettes) were sold in the United States using this design with advertising that emphasized the "health protection" supposedly provided by the filter.

Several packages of the 1952-1956 vintage Kent® cigarettes were obtained in their original sealed packaging and analyzed by Polarized Light Microscopy (PLM), Scanning Electron Microscopy (SEM), and Fourier Transform Infrared microspectrophotometry (micro-FTIR). The outer paper wrapper on the filter consisted of a white paper layer and a tan coating layer. The white paper was part of the continuous white paper that covered the entire cigarette. The inside of the filter consisted of rolled crepe paper with loose fibrous material. Some carbonate particles were also present. Both the white paper and the crepe paper were consistent with chemically processed wood paper fiber. The loose fibrous material consisted of crocidolite, cotton, and cellulose acetate fibers. The composition of the filter in terms of approximate percent by weight of total filter were: outer paper (white paper and tan coating), 16%; crepe paper including carbonate, 62%; crocidolite asbestos, 6%; synthetic fibers (cellulose acetate), 4%; and cotton fibers,12%. A corporate document described the Micronite® filter as containing cotton, crepe paper, cellulose acetate fibers, and approximately 7% to 25% crocidolite asbestos.[1]

The samples were examined for evidence of deterioration. No signs of mold, insect attack, or other deterioration were found. The crepe paper was found to be very flexible and had not become brittle with age.

Arrangements were made to test for particle release from regular and king-size Micronite® filter Kent® cigarettes using a standard smoking machine following generally accepted International Organization for Standardization (ISO) and Canadian smoke testing protocols.[2,3] A non-asbestos Kent® cigarette (later vintage) was smoked as a control. The smoke was collected on glass fiber Cambridge® filters. The filters were prepared for Transmission Electron Microscopy (TEM) using an acid/base digestion of the glass filter fiber that does not affect amphibole crocidolite fibers.[4] The samples were analyzed using American Society for Testing and Materials (ASTM) Standard Method D6281 for asbestos.[5] Crocidolite asbestos fibers were found to be released in the smoke of both regular and king-size Kent® Micronite® filter cigarettes.

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Microscopy, Kent® Cigarettes, Asbestos

D17 The Potential of Comprehensive Gas Chromatography (GC) in Forensic Fire Investigations

Martin Lopatka, MSc, Science Park, Amsterdam, CANADA; Gabriel Vivó-Truyols, Science Park, Amsterdam, SPAIN; Marjan J. Sjerps, Laan van Ypenburg 6, The Hague 2497GB, NETHERLANDS; Peter J. Schoenmakers, Science Park, Amsterdam, NETHERLANDS; Arian C. van Asten, PhD, Netherlands Forensic Institute, Laan van Ypenburg 6, The Hague, Zuid Holland 2497GB, NETHERLANDS; and Andjoe A.S. Sampat, MSc, Science Park, Amsterdam, NETHERLANDS*

After attending this presentation, attendees will have insight into the potential use of comprehensive chromatography for forensic applications as well as its limitations. Attendees will be able to assess whether comprehensive 2D GC (GCxGC) and comprehensive GCxGC/Mass Spectrometry (MS) will meet their case work needs and make a valuable contribution to their forensic laboratory.

This presentation will impact the forensic science community by illustrating how detailed chemical information as revealed by comprehensive chromatography can lead to new dimensions in chemical analysis of forensic evidence.

The COMprehensive FORensics (COMFOR) project is a collaborative effort between the University of Amsterdam and the Netherlands Forensic Institute to develop forensic methods based on comprehensive GCxGC and GCxGC/MS. Comprehensive chromatography has been successfully applied in many areas, including the petrochemical and food industry; however, its potential has not been fully exploited in forensic science. The multidimensional separation principle and modulation process lead to enhanced separation power, peak capacity, and sensitivity. This creates new opportunities when performing targeted trace compound analysis, broad untargeted screening, class characterization, and chemical comparison of complex samples of forensic interest.

Chemical analysis in forensic fire investigations deals with complex and highly variable fire debris samples in which the presence of minor residues of ignitable liquids may be indicative of arson. As ignitable liquids are often oil distillates of complex composition, the use of GCxGC and GCxGC/MS is especially promising in the field of forensic fire investigations. In this presentation, the latest results of the COMFOR project will be presented. This includes a detailed study into the chemical variation of white spirits in the Dutch market as analyzed by GCxGC and GCxGC/MS. Despite the very complex chemical composition, production and distribution conditions are such that chemical variation at a given point in time is actually quite limited for different white spirit brands; however, within-class differentiation is still feasible through detailed comprehensive analysis in combination with data processing and chemometric tools. Additionally, small-scale fire experiments were developed and conducted to efficiently generate realistic fire debris samples under controlled conditions. Fire debris head space was sampled on carbon traps which were extracted with DCM. The DCM extracts were subsequently analyzed with GCxGC and GCxGC/MS to detect ignitable liquid residues.

Current research focuses on the use of comprehensive chromatography in combination with chemometric methods and forensic statistical data analysis to classify ignitable liquid residues with greater objectivity. Finally, this project explores the possibly of within-class differentiation of ignitable liquids in fire debris samples. The COMFOR research shows the benefit of chemists, chemometricians, and forensic statisticians working together to ensure state-of-the-art chemical techniques to be applied in forensic practice and resulting in an assessment of evidential value.

The COMFOR project is funded by the Netherlands Organization for Scientific Research (NWO) by a grant.

Forensic, Fire, Investigation

D18 Physical Evidence Used in Rollover Crash Reconstruction

Kurt D. Weiss, MS, Automotive Safety Research, 5350 Hollister Avenue, Ste D, Santa Barbara, CA 93111-2326*

After attending this presentation, attendees will be able to catalog and illustrate physical evidence on the vehicle exterior and roadway commonly used in the reconstruction of rollover crashes.

This presentation will impact the forensic science community by identifying artifacts that are often used in determining rollover crash parameters such as roll direction, rollover count, and vehicle orientation at ground impact; examples will be drawn from real-world collision investigations.

Collision reconstruction is the scientific process of investigating, analyzing, and drawing conclusions about the causes and events of a vehicle collision. Commonly occurring crash categories are head-on, rear-end, intersection, T-bone, sideswipe, and rollover. Each crash type creates a unique set of physical artifacts related to the pre- and post-impact vehicle dynamics. Investigation of these collisions requires techniques that draw upon the quantity and quality of the observed and documented physical evidence. The physical evidence commonly observed in rollover crashes is presented and discussed.

Physical evidence used in the investigation and reconstruction of rollover crashes can be placed into two categories: (1) roadway evidence; and, (2) vehicle evidence. Additionally, roadway evidence can be divided into two subsets: (1) pre-rollover evidence; and, (2) evidence deposited during the rollover event. Examination of the vehicle may reveal evidence to suggest a roll direction or rollover count, but combining these vehicle artifacts with those observed at the collision site will yield a reconstruction of increased accuracy.

Pre-Rollover Evidence: (1) tire friction marks identify a vehicle's heading and orientation on the roadway before it rolls over. These rubber deposits are created because the tires can no longer generate the required lateral force and the tires begin to slide; and, (2) furrows are much like tire friction marks in that they establish the vehicle's path prior to the rollover event, but they are on dirt or vegetation and not on a paved roadway. A sliding tire cuts into and begins to plow the soil, creating furrows.

Evidence of Vehicle's Rollover Trajectory: (1) the trip point identifies the location where the vehicle stops sliding and starts to roll over. Oftentimes, the trip point is at the end of the furrows or tire friction marks, but sometimes an object, such as a curb, can be the tripping mechanism; (2) gouge marks are created when the vehicle has impacted or slid on the roadway. Depending upon the part of the vehicle that contacts the pavement or soil, these gouge marks will have a unique set of characteristics; (3) wheel imprints on the roadway will have a crescent shape corresponding to the wheel rim curvature. The orientation of these imprints relative to the roll path direction in conjunction with knowing the rollover leading side will determine if they are left or right side wheels; (4) fractured glass is commonly observed along the vehicle's roll path because tempered glass will break under vehicle structural deformation or by interaction with objects such as rocks. Knowing the color (clear or tinted) and thickness of the glass can help establish the window-breaking sequence during the rollover event; (5) paint, plastic, and rubber transfers occur when the vehicle has translational velocity relative to the ground and, by virtue of this sliding mechanism, paint, plastic, or rubber of the component making contact is thereby transferred; (6) component debris field can be found along the rollover path and varies with regard to vehicle type and roll distance. This field can include side view mirrors, roof racks, trim, bumper fascias, hubcaps, and vehicle contents such as handbags, blankets, and clothing; and, (7) the point of rest marks the end of the rollover event. If the vehicle does not rest on its wheels, dimple-like impressions may be observed on the vehicle exterior.

Vehicle Exterior: (1) scrapes on the vehicle exterior are indicative of ground contact. The orientation of unique collections of scrapes provides evidence for the roll count. Long scrapes suggest higher translational velocity relative to the ground; (2) wheel assemblies can reveal scrapes, gouges, or fractures. The side of the vehicle where these wheels reside will aid in determining vehicle orientation leading to and throughout the rollover phase of the collision sequence; (3) material flow to plastic components occurs when these vehicle parts impact the ground. Along with abrasions, if contact is sustained, material temperature can increase resulting in material flow in a direction opposing the vehicle's roll direction; and, (4) vegetation can become trapped in the wheel rim-tire bead junction. Observation of this material can indicate whether the vehicle rollover occurred on or off the pavement.

Thorough examination of physical evidence observed on the vehicle exterior and roadway will assist the investigator in a more accurate collision reconstruction. Commonly observed physical evidence is presented.

Physical Evidence, Rollover Reconstruction, Vehicle Artifacts

D19 Biomedical Engineering Contributions in the Analysis of Head and Brain Impact With Legal Perspectives by Counsel for the Department of Transportation: Bicycle vs. Auto, Seatbelts, and Motorcycle Accidents

Laura L. Liptai, PhD, BioMedical Forensics HQ CA/FL, 1660 School Street, #103, Moraga, CA 94556; and Landa S. Low, JD*, California Department of Transportation, 111 Grand Avenue, Ste 11-100, Oakland, CA 94612*

After attending this presentation, attendees will have a better understanding of how biomedical engineering principles apply to the engineering analysis of head and brain impact injuries.

This presentation will impact the forensic science community by demonstrating the importance of biomedical engineering analysis of head and brain impacts.

In the forensic analysis of a head or brain impact incident, the biomedical engineer is uniquely situated to offer insights with respect to the quantification of forces and accelerations. Because of the multidisciplinary aspects of most head and brain injury incidents, the biomedical engineer is able to tie the engineering aspects together with the medical diagnoses by the health care providers and forensic pathologists. Various head impact scenarios are analyzed from an engineering perspective and supported with analysis of physical evidence and/or experimentally verified test data.

Experiments conducted follow Federal standards using data acquisition software at 10,000Hz with a 4th-order Butterworth filter with a 1,650Hz cutoff frequency, per the Society of Automotive Engineers (SAE) J211. These experiments used different impact surfaces, including asphalt, and vehicle sections. Impact speeds were determined and replicated using an inverted pendulum impact protocol. Anthropometric crash test dummy head-forms and necks were used to study the multi-axial direct contact to the respective impact surfaces in both helmeted and unhelmeted modes in which the anthropometric sections are instrumented with triaxial piezoelectric ICP® accelerometers.

The legal perspective is provided for three cases involving different mechanisms of injury: helmet, occupant restraint (seatbelts), and bicyclist head impact. Counter arguments are presented to provide a complete perspective of the litigation issues. Also presented will be how the issues changed prior to trial that, in one case, changed the course of the trial itself.

The case studies for this presentation involve various allegations over the use (or non-use) of various mechanisms to prevent injury or death: seatbelts, motorcycle helmets, and bicycle helmets. The biomedical engineer's analysis sheds light on both liability and damages issues, presents the factors that caused the accident and the resulting injuries, and discusses whether the injury would be reduced or eliminated with the proper use of a restraint device or helmet.

The case background, forensic questions, and brief results are summarized below for each of the three cases to be discussed. The presentation will include discussion of the engineering approach, fundamental principles employed, methods, results, and conclusions.

Seatbelt Analysis: a woman and her husband pulled out to make a left turn onto a rural two-lane highway, directly into the path of a truck, resulting in a relatively low-speed impact. The husband in the passenger seat died as a result of a head/brain injury. Plaintiff argued that her husband was wearing a seat belt, but nonetheless suffered a fatal injury. The defense argued that he was not wearing a seatbelt and would likely have survived the accident if he had been wearing one. A careful analysis of the trauma clearly revealed the mechanism of injury resulting in this man's death. This case went to trial.

Solo Motorcycle Accident: a young woman was a passenger on a motorcycle when the rider, who was going uphill on a curve, struck a guardrail, ejecting his passenger. She struck something with her face, which caused massive facial trauma. Plaintiff alleged she hit a nail sticking out of the stop sign post. But, could it have been a guardrail post, the ground, or something else? Biomedical engineering analysis determined the mechanism of injury. The matter settled.

Bicycle vs. Auto Accident: an un-helmeted bicyclist was struck by a car as he crossed a freeway on-ramp. He was thrown over the car and struck his head on the asphalt, suffering a severe traumatic brain injury. Plaintiff argued that he was not astride his bicycle, but was riding it as a scooter, with his right foot on the left pedal. Because he was not technically "riding," he did not legally have to wear a helmet. Defense argued that the injuries would have been prevented or significantly lessened if he had been wearing a helmet. Careful biomedical engineering assessments pieced together the physical evidence, some of it missed by both the accident reconstruction and bicycle experts, to determine how the injury occurred. An experiment then quantified the accelerations with and without a helmet. The matter settled.

Biomechanics, Brain, Impact

D20 Non-Collision Moving Vehicle Fire Caused by Escape of Exhaust Heat and Combustion Gases Due to Muffler Design and Materials Defects

Mark C. Pozzi, MS, Sandia Safety Sciences, 2 Marietta Court, Ste A, Edgewood, NM 87015; Dean L. Jacobson, PhD, 4665 S Ash Avenue, Ste G4, Tempe, AZ 85282; David Bosch, PhD, 4665 S Ash Avenue, Ste G4, Tempe, AZ 85282; and Scott Anderson, BS, 4665 S Ash Avenue, Tempe, AZ 85282*

After attending this presentation, attendees will understand how a wide range of forensic investigation and analysis techniques, ranging from fire cause and origin to metallurgical analysis of materials and welding to analysis of how and why fire can spread rapidly, can be employed.

This presentation will impact the forensic science community by describing the methodology utilized to investigate a non-collision fire involving a moving vehicle which had demonstrated no known pre-fire operational defects or damage.

A two-door utility vehicle in original equipment manufacturer (stock) condition ignited and rapidly burned while being driven at normal highway speed on a paved road. There was no evidence of a collision, other pre-fire vehicle damage, or operational malfunction of seats or doors. Despite immediate rescue efforts by the initially uninjured driver, two conscious, initially uninjured children restrained in safety seats in the rear seat of the vehicle died due to the extremely rapid spread of the fire. Forensic examination of the vehicle showed a burn pattern consistent with a fire originating from the carpeted interior floor pan area between the front and rear seats. The fire then spread via burning of the adjacent front and rear seats. There was a corroded hole in the floor pan directly below the area of fire origination. Below the floor pan hole was the muffler, which showed clear evidence of long-term pre-fire deterioration, including a missing heat shield. This allowed exhaust gases to heat, corrode, and eventually penetrate the floor pan and the flammable interior components above the hole. The muffler utilized a combination of poor design, inferior construction techniques, and inferior materials that caused or contributed to the escape of hot, corrosive exhaust gases, as well as allowed those gases to strike the unprotected floor pan for an extended period of time. Because the hole in the floor pan was covered by carpeting and insulation, these defects were not noticeable to the operators of the vehicle prior to ignition.

Vehicle interior materials were apparently certified by the manufacturer to be in compliance with the flame resistance requirements of Federal Motor Vehicle Safety Standard (FMVSS) 302; however, these materials were not capable of handling the prolonged heating caused by the hot exhaust gases, particularly when applied to the underside of the padded carpeting. This is not a heat vector typically anticipated by FMVSS 302, but it is certainly foreseeable given the close proximity to hot exhaust components. Once ignition conditions were reached, the spread of the fire was extremely rapid. Despite immediate attempts to remove the two rear-seated occupants, they both perished in the fire.

There have been limited research studies on the effects of exhaust system heat, particularly that generated by catalytic converters or mufflers, causing fires related to flammable materials such as dry vegetation, dead leaves, and paper on the ground under parked or running vehicles; however, this case involves a type of exhaust system failure and adjacent vehicle structural/materials failure which is substantially different. The extremely rapid onset of fire illustrates that vehicle interior materials may be far more dangerous when subjected to some types of foreseeable ignition sources than were anticipated by FMVSS 302.

The extremely rapid spread of fire in the interior of a vehicle that was supposedly compliant to FMVSS 302 is also notable. Based on available information, this type of fire normally does not result in a loss of life, therefore it likely does not receive as much attention from automotive manufacturers or safety regulators as other types of vehicle fires. Emergency egress from a vehicle, particularly rear seated occupants, is a commonly overlooked aspect of passenger protection.

The educational objectives of this presentation will be relevant to forensic practitioners in the engineering sciences, fire and arson investigation, vehicle design, accident investigation, criminalistics, jurisprudence, and pathology, with potential interest to those in toxicology and general forensic sciences.

FMVSS302, Non-Collison, Fire

D21 Refueling Fire Caused by Defective Fuel Pump Nozzle, Electrostatic Discharge Ignition, and Violations of Safety Practices

Mark C. Pozzi, MS, Sandia Safety Sciences, 2 Marietta Court, Ste A, Edgewood, NM 87015*

After attending this presentation, attendees will be able to demonstrate how proper forensic investigation can determine, by using the scientific method, how to conclusively prove fire cause and origin, and how it relates to injury causation, property damage, violation of safety procedures, and related issues.

This presentation will impact the forensic science community by illustrating the methodology for investigating an emerging fire hazard, describing how to investigate a catastrophic refueling fire, and demonstrating how the source of fuel leakage and ignition was determined and how other sources of ignition were eliminated.

This presentation involves a forensic investigation of a gasoline refueling fire at a self-serve station. Multiple violations of safety practices before and during the fire caused or contributed to the fire. These violations caused a known defective fuel pump nozzle to remain in service with no warnings to consumers or lock-out/tag-out precautions. Documented failures of the nozzle earlier on the day of the incident were ignored by the operators of the refueling station. When utilized to fill a vehicle in the normal manner, the defective filler nozzle failed to shut off, spilling a large amount of gasoline from the filler neck onto the ground as the pump continued to operate. This falling fuel was capable of generating significant static electricity. No emergency shut-off switch was available near the gasoline pump. The vehicle passenger attempted to manually shut off the nozzle without success. The driver began to exit the vehicle to deal with the leaking fuel. When the driver rotated and slid off the seat and then touched the ground, an electrostatic discharge was generated sufficient to ignite the vapors spreading from the overflowing gasoline. A catastrophic fire resulted, which caused severe burns to the driver, destroyed the vehicle and refueling pump, and also destroyed much of the gas station. The station attendant failed to observe the refueling operation and failed to shut off the fuel pump even when aware of the fire. This caused the pump to continue to supply full-flow pressurized fuel to the fire for several minutes. Evidence of defects in the refueling nozzle were discovered; these defects caused the nozzle to malfunction, preventing proper shut-off. Claims that the fire had ignited because the driver had left the engine running were disproven via forensic inspection of the engine and components of the ignition system. There were no contributions to the fuel leak or fire by the vehicle or its occupants. Also, claims made that evidence spoliation had occurred as a result of the forensic investigation were disproven by the use of on-scene and subsequent photographs and other data. A previous similar incident at a different station operated by the same company was also uncovered, which had not been disclosed.

Refueling fires which were ignited by Electrostatic Discharge (ESD) ignition have been known for many decades. There have been approximately 200 reports of ESD ignition related to ground vehicle refueling fires in the United States since the late 1990s, with initial suspicion that cellular telephones were responsible; however, ESD was the actual cause. This has become a recognized significant hazard resulting in warnings being posted on many refueling pumps. ESD has caused fires in both ground vehicle and aircraft refueling, and it can occur with propane as well as liquid fuels.

Refueling, Defective, Electrostatic Discharge

D22 Friction Tire Testing of a Run-Flat Condition Sport Utility Vehicle (SUV) Tire

Kurt D. Weiss, MS, Automotive Safety Research, 5350 Hollister Avenue, Ste D, Santa Barbara, CA 93111-2326; and Jacqueline Paver, 501 Meigs Road, Santa Barbara, CA 93109*

After attending this presentation, attendees will better understand the value of dynamic tire friction testing as a tool for collecting data that relates tire inflation pressure to the rolling friction of a run-flat SUV tire.

This presentation will impact the forensic science community by providing data for speed calculations at the start and end of tire friction marks. This data will help traffic collision reconstructionists understand the effect of tire inflation (e.g., the run-flat condition of a rear tire) on vehicle (and occupant) kinematics and dynamics. These results are also important to biomechanical engineers, because the literature documents increased likelihood of vehicle instability and rollovers, and increased likelihood and severity of injury as a result of loss of tire pressure and/or failure.

Collision Overview: On a desert highway, the right rear tire of an SUV catastrophically failed at approximately 70mph. Pre-rollover tire pressure loss resulted in an unintended vehicle heading change. Right oversteering driver input resulted in clockwise yaw, but then left oversteering driver input resulted in counterclockwise yaw. Front tire friction marks were documented. The SUV traversed down a dirt and rock embankment. The SUV flipped, passenger-side leading, at about 40mph and rolled uphill 3¼ times over a distance of approximately 125ft. The SUV came to rest on its wheels.

The female driver sustained fatal head, chest, and abdominal injuries with extensive musculoskeletal fractures. The left second-row, ejected male passenger sustained diffuse axonal brain injury, neck fracture, and chest injuries with residual impairment. These injuries most likely occurred inside the vehicle or during ejection.

Tire Analysis: The subject vehicle and failed tire were inspected by tire experts who agreed with the sidewall failure mechanism (i.e., a complete circumferential separation of the outer sidewall from the tire casing due to operation while severely underinflated). A tear observed on the failed tire inner sidewall adjacent to the tread belt was hypothesized as the source of the air pressure loss. In this instance, it was unknown how long it took the tire to deflate and cause the outer sidewall failure.

Dynamic friction testing was conducted with an exemplar SUV equipped with a new 275/60R16 tire installed on the right rear wheel. Tests were performed on a rough and weathered asphalt roadway. The test vehicle was accelerated to a speed of 39mph to 53mph and then allowed to coast (i.e., without brake application) to speeds between 16mph and 22mph. The test matrix included two test runs each for both directions of the roadway. A baseline test of the vehicle's overall rolling friction (including air resistance) was determined with all tires inflated to 38psi. Then, the air pressure of the right rear tire was bled out to 20psi, 10psi, and about 3psi, respectively. The four test runs and resulting deceleration rates were averaged for each test tire pressure. Vehicle speed was measured at 20Hz with a Racelogic VBox II Lite.

The test tire was documented with: (1) an exterior-mounted video camera that captured the tire flex and road noise; and, (2) an on-board video camera that captured steering wheel and operator motion due to the low-pressure right rear tire.

Results: The test data was analyzed using the VBox Tools software. The overall road friction (including air resistance) of the test SUV was -0.0263g at 38psi and 20psi, -0.0325g at 10psi, and -0.0435g at about 3psi. Despite the rough and weathered test roadway asphalt, the steering wheel did not appear to demonstrate significant rotation or vibration as a result of the low pressure in the test tire; however, the noise level recorded by the external video camera, as well as that detected by the test vehicle operator, was remarkably loud inside the vehicle cabin. Surprisingly, the outer sidewall temperature of the test tire with about 3psi was too hot to touch immediately after testing. Test results demonstrated the value of dynamic friction tire testing and the results show that overall road friction increases with decreasing tire pressure.

Run-Flat, Friction, Rollover

D23 Witness Identification Under Low Light-Level Conditions: A Case Study

James B. Hyzer, PhD, Hyzer Research, 1 Parker Place, Ste 330, Janesville, WI 53545-4077*

After attending this presentation, attendees will be aware of a case study in which the defense in a capital murder case unsuccessfully argued that the defendant shooter was not able to identify his victim as a police officer under the low light-level conditions that they alleged existed at the scene of the crime.

This presentation will impact the forensic science community by increasing awareness regarding nighttime visibility, mesopic vision, photometry, illumination, and the pitfalls associated with trying to use photography as evidence to demonstrate to a jury what an individual could or could not see under low light-level conditions.

The subject case involves the shooting death of Officer Travis Murphy by defendant Danny Martinez in a Phoenix, AZ, residential neighborhood at approximately 1:30 a.m. on May 26, 2010. A defense argument was that the defendant did not know he was shooting a police officer because of the low light-level conditions that existed when he fired his shots. In his report, one of two visibility and illumination experts for the defense stated the following: "Therefore, based on my measurements and observations and with reasonable scientific certainty, I believe that the shooter could have had difficulty in visually determining whether the decedent was a police officer because of low light levels and because of the disability glare from either flashlight that the decedent might have used." The bases for his opinion are photometric measurements and observations at a recreation of the crime scene in February 2015. This expert additionally made two sets of four bracketed-exposure images from both the vantage point of the shooter and of the officer. Referring to an exhibit of two photographs in his report, he stated: "I selected the most realistic exposure from the four exposures for each scene for this exhibit."

An expert witness was retained by the prosecution to review and possibly rebut the analysis and testimony of the defense experts. Subsequent to a pre-trial motion to exclude the defense expert's nighttime photographs, they were withdrawn as evidence. Testimony by the prosecution's expert witness at a pre-trial hearing and as a rebuttal witness at trial concentrated mainly on changes in measured illuminance levels that occurred between the time of the crime in 2010 and both experts' separate inspections in early 2015, as well as the contribution of a 97% full moon that was visible on the night of the crime.

The measured illumination levels and a description of the level of detail that can be seen by an observer under low levels of illumination was an important factor in helping the jury decide whether or not the defendant could identify his victim as a police officer.

Witness Identification, Visibility, Photography

D24 Forensic Engineering Investigation of a Dual Fatality Auto-Pedestrian Collision by an Impaired-Vision Driver

Adam Aleksander, PhD, Aleksander & Associates, PA, PO Box 140558, Boise, ID 83714*

After attending this presentation, attendees will be informed of details of the human visual system that were overlooked by the original law enforcement investigation and that help to explain the technical backdrop to a tragic dual fatality auto-pedestrian collision at a marked crosswalk.

This presentation will impact the forensic science community by discussing trial strategy and the use of witnesses, in addition to the final sentence, consequences for the driver, and implications for driving with impaired vision.

At about 9:30 a.m. on August 19, 2009, an elderly couple, 78 years and 76 years of age, went on their daily walk on the route they followed every day. This included crossing a four-lane suburban road in a residential area. Visibility was unlimited, the weather was clear and sunny, and there was no reason to expect a tragic end to their walk.

At the same time, a 55-year-old male, who was a regional representative for an insurance company and administered many types of accident and litigation claims, left his residence on a seemingly routine drive to his office. A few minutes later, as the couple crossed the street, they were hit in the crosswalk. One died at the scene, the other shortly thereafter at the hospital. The driver pulled over and waited for police. Law enforcement analyzed the location, probable speed, and perception reaction time, in addition to some unexplained behavior at the scene. The driver was charged with the two homicides and driving under the influence; however, he had not drunk any alcohol, but had taken a Benadryl medication, as was prescribed for a chronic illness. Approaching trial, he faced two 15-year terms.

However, the medical record of the driver offered some insight into the collision. He had experienced several brain tumors and surgeries. One of these surgeries inadvertently cut the optic nerve, resulting in total blindness in the right eye and partial loss of vision in the left eye. In fact, he retained only his temporal vision.

The presentation will discuss the details of the visual system, the nasal and temporal visual fields, and why the state of Idaho licensed this individual to drive under these conditions. Perception psychology also comes into play in this instance as humans have remarkable abilities to compensate for visual and sensory deficits.

These considerations were useful in understanding the sequence of events that led to the fatal collision. In fact, the driver saw the couple from a distance, reported that they waved to him to go through the intersection, then lost sight of them until the actual collision, not due to drug impairment, but due to the nature of the visual deficit. The reported wave-through action by one of the decedents had a credible basis and will be discussed. Trial strategy and the use of witnesses will also be discussed as well as the final sentence, consequences for the driver, and implications of driving with impaired vision.

Optic Nerve, Impaired Vision, Crosswalk

D25 A Case Against "Inattentive Driving" as a Cause for Some Nighttime Vehicle Pedestrian Accidents

James B. Hyzer, PhD, Hyzer Research, 1 Parker Place, Ste 330, Janesville, WI 53545-4077*

After attending this presentation, attendees will be aware of a case against "inattentive driving" as a cause for a number of nighttime vehicle pedestrian accidents.

This presentation will impact the forensic science community by explaining that some drivers exercising reasonable care with respect to lookout can and will miss visually perceiving pedestrians in their path under nighttime driving conditions.

A common and obvious cause of numerous roadway accidents is the driver not seeing a pedestrian in time to respond and avoid collision. It is not uncommon for drivers who have hit pedestrians, for example, to report that they "heard the thump but never saw him" or that they "didn't see him until it was too late." Accident reports commonly attribute such claims to "inattentive driving," presumably based on an assumption that a driver who doesn't see a pedestrian who must have been visible couldn't have been paying attention. The job of the expert, then, is to determine whether the signal value of the struck pedestrian was sufficient relative to the driver's visual field to assuredly capture the attention of all alert drivers exercising reasonable care to enable them to respond in time to avoid collision. Through a critical review of published scientific literature, it will be shown that it does not follow that simply because a pedestrian is visible to some drivers, he will necessarily be recognized as a hazard and responded to in time by all drivers exercising reasonable care with respect to lookout.

To correctly express these concepts to a jury, it is important to understand the relevant terminology as it relates to visibility of pedestrians. To *detect* a pedestrian means to discover or determine that something is there; however, a *detected* pedestrian may not necessarily be *recognized* as a pedestrian. To *see* the pedestrian simply means to *perceive* him by the eye or by vision. A pedestrian is *visible* if he is capable of being seen. A pedestrian is *conspicuous* if he attracts or tends to attract the attention of an observer who may not be looking for him. Conversely, a pedestrian is *inconspicuous* if he is not readily noticeable or discoverable by vision. The term *conspicuity*, then, is the capacity of an object, such as a pedestrian, to be readily discovered by vision. Therefore, even though a pedestrian may be in plain sight and visible, he must be sufficiently conspicuous relative to his surroundings to be seen in time by all drivers. At the two extremes, pedestrians that are highly conspicuous should be seen at the greatest possible distances and pedestrians that are highly camouflaged may not be seen or recognized at all.

This presentation offers a case study in which the facts and evidence are inconsistent with the alleged cause of "inattentive driving." It will be shown that in some cases, drivers will miss visually recognizing pedestrians who first become visible at a distance too close to avoid impact because the driver is reasonably paying attention to a location farther ahead in his path and is looking for hazards that he does have time to avoid.

Accident Reconstruction, Visibility, Inattentive Driving

D26 An Engineering Perspective on Case Studies Where Performance Does Not Match Scientific Predictions — The Expansive Nature of Collapsible Soils and Other Engineering Oddities

Michael D. McDowell, MS, 24665 E Ontario Place, Aurora, CO 80016*

After attending this presentation, attendees will be able to: (1) describe how soils issues relate to construction defect claims; (2) describe the challenges in accurately predicting soil behavior; (3) describe potential mistakes made by parties assessing the nature of soil movement; and, (4) present multiple case studies which illustrate engineering oddities and challenges.

This presentation will impact the forensic science community by describing some common challenges when assessing construction defect cases involving soil movement and by improving the understanding of engineering principles which can be applied in the field and laboratory to more accurately evaluate construction claims involving such movements.

Soils-related issues are among the most common allegations described in construction defect claims today. Generally, soils-related allegations include inadequate grading and drainage and questionable soil compaction. The more expensive and troubling allegations generally include foundation modification based on the presence of expansive or collapsible soil. Less common but equally challenging allegations include perched groundwater conditions and corrosive soils. These issues have been documented as being a significant contributing factor to financial demands in construction defect claims. Case review of several hundred construction defect claims has shown that alleged damage related to soils issues constitutes several hundred million dollars in recommended repairs.

Because questionable structural performance may be attributable to a variety of soils-related issues, it is important to adequately assess the composition and behavior of soil in a laboratory. Likewise, it is important to compare one's hypothesis against the performance of the structure (and vice versa). Understanding the nature and predicted performance of soil is generally the first step in a forensic evaluation.

Experience has shown that observational evidence or performance of a structure alone is generally insufficient to provide a scientifically supportable opinion. This is particularly true when formulating opinions on causation or potentially responsible parties. In some cases, settlement of soils may be attributable to design-related issues (e.g., hydro-collapsible or compressible soils) and in other cases, settlement may be attributable to construction-related issues (e.g., inadequately compacted soil). While the term "soil settlement" may be an appropriate diagnosis, it is important to understand the underlying cause of the soil settlement since the observed condition may be attributable to the party who designed the project or the party who constructed it — or potentially both parties.

When assessing soil conditions and resultant structural performance, there are several challenges facing forensic engineers. In the case studies provided, examples will be presented in which the performance of a structure did not match the predictions or opinions offered by the forensic engineer. In a few instances, the performance of a structure was the opposite of the predicted behavior, yet the forensic engineer maintained an unsupportable opinion throughout the course of litigation. The case studies provided will demonstrate that the consistent application of the scientific method will reduce the risk of erroneous opinions.

Structural, Performance, Defects

D27 A Structural Analysis of a Gymnasium Collapse Using the MIDAS Program

Chan-Seong Park, PhD, Division of Forensic Safety, National Forensic Service, 10 Ipchun-ro, Wonju-si, Gangwon-do 220-170, SOUTH KOREA; Jong-Heon Shim, MS, National Forensic Service, 10 Ipchoon-ro, Wonju, SOUTH KOREA; Jin-Pyo Kim, PhD, 1524 Yusungdaero Yusung-gu, Daejeon, SOUTH KOREA; and Nam-Kyu Park, PhD, National Forensic Service, 10 Ipchoonro, Wonju, SOUTH KOREA*

After attending this presentation, attendees will understand the feasibility of using the MIDAS program as a tool for the structural analysis of building collapses.

This presentation will impact the forensic science community by introducing an effective tool for the structural analysis of building collapses.

When analyzing a building collapse, it is imperative to perform a structural analysis; however, in most cases, it is difficult to model the collapsed building structure using general Finite Elements Method (FEM) tools within a restricted time. This study focuses on the effectiveness of the MIDAS program by introducing a case analysis report. As a case report, the structural analysis of the collapsed roof of a gymnasium is presented. The roof of the gymnasium under study was built as a truss structure and collapsed six hours after being built. The roof was supported on one side by hinge structures and on the other side by sliding structures. All the anchor bolts of SS400 carbon steel for the sliding supports fractured with bending. The fractured surfaces of the bolts show dimples without cleavages when examined using a Scanning Electron Microscope (SEM).[1,2] Mechanical properties of the fractured bolts, the hardness, the yield strength, and the ultimate strength were within or slightly above the specified range shown on the material specification when examined using the Advanced Indentation Tester (AIT). The center members of the roof fractured along the wall. The fractured surfaces of center members of the roof showed dimples without cleavages when examined using SEM.

A structure analysis using the MIDAS program was performed by modeling the collapsed roof structure. The MIDAS program serves as a structure wizard with material and section properties, making it possible to easily model the structure.[3,4] The structural analysis revealed that the stress of the center member of the roof exceeded the yielding stress because of sliding supports, which were the main cause of the collapse.

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Structural Analysis, Building Collapse, MIDAS Program

D28 Forces Transmission to the Skull in a Case of Mandibular Impact

Lucile Tuchtan, MD, 63 Chemin Des Aurengues, Marseille 13013, FRANCE; Marie-Dominique Piercecchi-Marti, PhD, 264 Rue Saint Pierre, Marseille 13005, FRANCE; Christophe Bartoli, MD, 264 Rue Saint Pierre, Marseille, FRANCE; Pascal Adalian, Laboratoire ADES, Marseille, FRANCE; Georges Leonetti, PhD, 264 Rue Saint Pierre, Marseille, FRANCE; and Lionel Thollon, 264 R St Pierre, Marseille, FRANCE*

After attending this presentation, attendees will better understand direct mandible impact, the level of energy required to create a mandible fracture, and the energy dispersion to the skull and to the brain.

This presentation will impact the forensic science community by providing a better understanding of the force transfer mechanisms into and from the mandible.

Background: Forensic investigations have been reported regarding the loss of consciousness and cardiac arrests resulting from direct mandible impact; however, the mechanisms by which the forces are transferred to the skull through direct mandible impact remain unclear.[1] A study was conducted regarding direct mandible impact on the level of energy required to create a mandible fracture and on the energy dispersion phenomenon to the skull and to the brain.

Materials and Methods: This study combines an experimental and numerical approach. Mandible strike was studied using experimental trials performed on postmortem human subjects. A finite element model of the head and face of a male was also developed based on tomodensitometry scans. The model was validated with literature data and experimental trials. A parametric study was then performed to study the effect of diverse variables such as the dentition integrity, cortical bone thickness, etc.

Results: The forces measured on the reference model were 3,000N on the chin, 1,800N at the condyles, and 970N in the occiput. Of all the results, a decrease of approximately one-third of the efforts from the chin to the base of the skull and a lower half of the still forces at the occiput was observed, except in the edentulous and for the lateral and frontal impact where the force is transmitted directly to the skull base area.

In this study, the skull model was validated to demonstrate the energy transmission to the skull, but the focus was the von Mises stress distribution of the cerebral pressure areas. The von Mises stress was distributed with an anterior-to-posterior orientation through the temporal lobe and ended at the brainstem level. Viano et al. found that a hook strike increases the stress in the temporal region and at the midbrain level.[2] Belingardi et al. developed a Finite Element Method (FEM) of the head based on the experimental trials performed by Nahum et al. and found increased cerebral pressure with significant stress in the coronal section of the brainstem, which confirms its pivotal role in movement of the brain.[3,4]

Thus, it can be supposed that during an uppercut, part of the impact energy reaches the brainstem, which is obviously supported by the levels of stress found at the condyles during the simulations and the fractures observed at the temporomandibular joint in both experiments. This result is interesting from a forensic point of view because the pressures observed in the brainstem could lead to a vagus nucleus stimulation, which may be involved in cardiac arrest via the cardio-inhibitory reflex.

Conclusion: A 3D model of the mandible and face bones was created for this study to better understand the force transfer mechanisms into and from the mandible. The parameters of the model may be modified to suit the individual characteristics for forensic investigations and legal matters.

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Investigations, Mandible, Uppercut

D29 Forensic Engineering Examination of Stucco on a Concrete Masonry Unit (CMU) Wall, Paint Layer Evidence, and Crack Propagation

Adam Aleksander, PhD, Aleksander & Associates, PA, PO Box 140558, Boise, ID 83714*

After attending this presentation, attendees will be informed of a basic method of determining the ordering and timing of paint layer application on a stucco surface applied to a CMU wall structure.

This presentation will impact the forensic science community by explaining how this method of examining the time-related ordering of paint deposition was critical to understanding the sequence of paint material application and subsequent fracture propagation through the structure.

Two basic methods have been used for centuries in the scientific and engineering examination of materials — surface examination and cross-section inspection.

Cross sections are commonly used in many disciplines, including engineering, material science, and medicine. For example, sections through welds or fractures are a primary means of metallurgical analysis. Similarly, Computed Axial Tomography (CAT) scans and Magnetic Resonance Imaging (MRI) techniques are now everyday terms and are, in effect, sophisticated cross-section methods to analyze the human body.

In this case, sections of stucco were removed, sectioned, polished, and magnified. A clear sequence of events was developed based on the physical evidence, namely identification of individual paint layers, the sequence of application, and the intrusion of paint into pre-existing fractures.

In spite of disproportionately vigorous and impassioned objections and motions *in limine* from opposing attorneys, this cross-section method was in fact grudgingly admitted at trial by the judge, and is described in this presentation, along with the illustrative exhibits and related graphics. Since the opposing attorney stridently objected to this method as being novel and unpublished, it is being presented through this presentation.

The underlying case concerned a substantial CMU structure that was originally built in September of 2001, and coated with a white primer paint approximately 30 days later. Subsequently, in the following months a brown pigment paint layer was applied. In the following years, an additional coat of a similar brown pigment paint was applied. In 2012, municipal road improvement construction along the wall was alleged to have created a network of fractures and cracks in the CMU wall. Furthermore, numerous learned reports were submitted by various experts alleging that the fractures could be explained by engineering formulations and other arguments. None of the reports effectively refuted the physical evidence that the fractures were in fact evident well before the date of the municipal construction, time stamped by the order of paint application; this evidence will be presented.

NACE International (formerly National Association of Corrosion Engineers) identifies tools and methods to examine paint thickness, but these methods are not applicable to this type of investigation. The method described in this investigation may be useful in similar future investigations.

Briefly described, the method was to adhere a 3”x3”x$^{1}/_{8}$” (75mm x 3mm) Fiberglass Reinforced Plastic (FRP) laminate with a generous layer of epoxy over a fracture line of interest. The FRP laminate was taped in place and allowed to cure for 24 hours. A diamond blade on a portable grinder was used to cut a 4” coupon from the stucco wall, removing the FRP laminate and the attached stucco layer (this also exposed the underlying prior fracture in the CMU). The FRP laminate preserved the geometric relationship in the stucco section and preserved the fracture features. The FRP surface was marked, photographed, then the entire sample was sectioned on a masonry diamond blade wet cutter. The sections were $^{3}/_{4}$” (20mm) thick, resulting in four sectioned samples per location.

Each cut section was then polished on a wet diamond lapidary surface. The resulting finished samples were then photographed and measured using both optical and digital microscopes. The resulting inspection and analysis clearly revealed the individual paint layers, allowed dimensional measurements, and illustrated the intrusion of paint into the pre-existing fractures long before the construction began near the CMU wall in question. This data will be presented.

CMU, Stucco, Paint Layer

D30 Fuel Gas Odorization: History, Requirements, Application, and Challenges for Natural Gas and Propane

Tim G. Dunn, MS, Dunn Laboratories, Inc, 230 Spring Ridge Trace, Roswell, GA 30076*

After attending this presentation, attendees will gain insight into the history of fuel gas odorization, present applications for odorizing natural gas and propane, and the challenges faced for the odorant to provide a means of warning.

This presentation will impact the forensic science community by providing an understanding of the issues pertaining to gas odorization.

For many forensic engineers, the first question following a fuel gas explosion or fire is whether victims or witnesses noticed a gas odor, and if not, why not? The practice of adding a malodorant to a fuel gas goes back to the 1880s, when ethyl mercaptan was used to odorize water gas — hydrogen/carbon monoxide. For many years, coal gas, which contained a substantial level of carbon monoxide, obviously making it very toxic fuel gas, was used in both Europe and the United States. From the 1950s to the mid-1960s, new transmission pipelines brought natural gas to various parts of the country, bringing with it more focus on odorization practices.

There were several studies conducted by the United States Bureau of Mines (BOM) from 1919 to 1931 that addressed the topic of warning agents for fuel gases. In the 1931 BOM study, other means of warning were considered, such as the use of irritants (eye, nasal) and sternutators (sneezing agents).[1] Many of the warning agents were limited by the fact that they were unsuitable due to toxicity or corrosivity. This study reached the conclusion that ethyl mercaptan was the most effective product for causing complaints of leaks; crotonaldehyde, an irritant, was determined to be the next best candidate. The necessity for a warning agent in fuel gases came to the forefront following the natural gas explosion at the New London/Texas High School, which occurred on March 18, 1937: 239 persons lost their lives from the results of a leak of unodorized gas piped into the building from a nearby oil field. The day after this accident, the State of Texas proposed the first law requiring that fuel gases be odorized. The requirement for odorizing propane came a short time later with the National Bureau of Fire Underwriters (NBFU) (the predecessor to the National Fire Protection Association (NFPA)) pamphlet 58.

The Natural Gas Pipeline Safety Act of 1968 delegated responsibility for regulation and monitoring of pipeline transport of gas to the Secretary of Transportation. The Office of Pipeline Safety was formed in 1968. In the Code of Federal Regulations, 49CFR Part 192.65, Odorization of Natural Gas specifies the following: "(a) A combustible gas in a distribution line must contain a natural odorant or be odorized so that at a concentration in air of one-fifth of the lower explosive limit, the gas is readily detectable by a person with a normal sense of smell." For propane, similar requirements were found in the Liquefied Petroleum (LP) Gas Code, National Fire Protection Association (NFPA) pamphlet 58: "All LP-Gases shall be odorized prior to delivery to a bulk plant by the addition of a warning agent of such character that the gases are detectable, by a distinct odor, to a concentration in air of not over one-fifth the lower limit of flammability." The challenge presented by these situations is to apply engineering principles to meet these performance standards!

This presentation will discuss the means by which natural gas and propane is odorized, describe the prominent odorants used today, and the challenges encountered for an odorant-based warning system. With propane, the presentation will discuss the 1977 testing conducted in the Bartlesville study, additional testing by the Institute of Gas Technology, now Gas Technology Institute (GTI), and testing performed with released fuel gas in Round Lake, MN.[2]

Due to the properties of fuel gases and the results of the testing, the conclusion reached is that electronic flammable gas detectors offer an added level of safety to the gas consumer.

Reference(s):

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2. United States Department of Energy, Bartlesville Energy Research Center study, *A New Look at Odorization Levels for Propane Gas*.

Odorization, Fuel Gas, Warning Agent

D31 Redesign of a StepMeter for Direct *In Vivo* Measurement of Barefoot Skin Friction

Marcus P. Besser, PhD, Pennsylvania State University Abington College, 1600 Woodland Road, Abington, PA 19001-3900; and Mark I. Marpet, PhD, PE, 14 Cowie Road, Chester, NJ 07930-9715*

After attending this presentation, attendees will understand some of the issues in barefoot tribometric slip-resistance testing, specifically assessment of barefoot slip resistance, and how current research is being conducted to address these issues and improve the biofidelity of these measurements.

This presentation will impact the forensic science community by allowing the collection of a cohort of data on barefoot subjects to characterize the subject-to-subject variation in barefoot pedestrian slip resistance on different surfaces.

Background: Testing of floor-surface slip resistance is routinely conducted using walkway tribometers. These devices use a sample of outsole material to test floor surfaces, either in the laboratory or *in situ*; however, none of these devices operates (or slips) in the manner of a human pedestrian. Approximately 15 years ago, to assess the biofidelity of tribometric floor slip-resistance testing, a custom StepMeter was developed.[1] This device assessed a human subject stepping onto a floor surface wetted with water. The device was later modified to test a seated subject, as that posture provided greater reliability as shown by a steeper logistic-regression curve.[2-4] In 2011, the American Society for Testing and Materials (ASTM) F2508 Standard Practice for Validation and Calibration of Walkway Tribometers Using Reference Surfaces methodology was adopted.[5] The StepMeter was validated using this protocol with a Neolite® Test Liner (NTL) test foot; however, assessment of barefoot slip with this device and the F2508 protocol identified differences between the inert test foot and *in vivo* subjects.[6,7] It is believed that these differences are intrinsic to the *in vivo* foot, but need to modify the StepMeter to be able to collect the range and quantity of *in vivo* data needed to characterize barefoot slip. This presentation will discuss the redesign of the StepMeter.

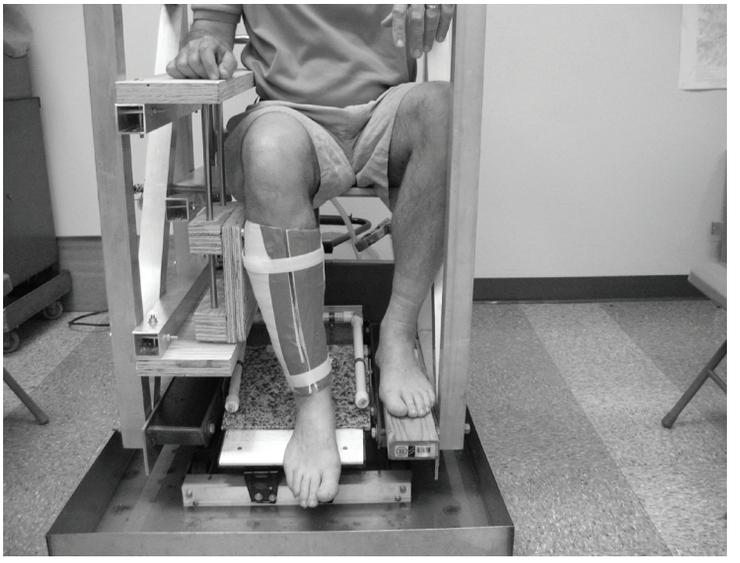

Figure 1: Seated subject in StepMeter

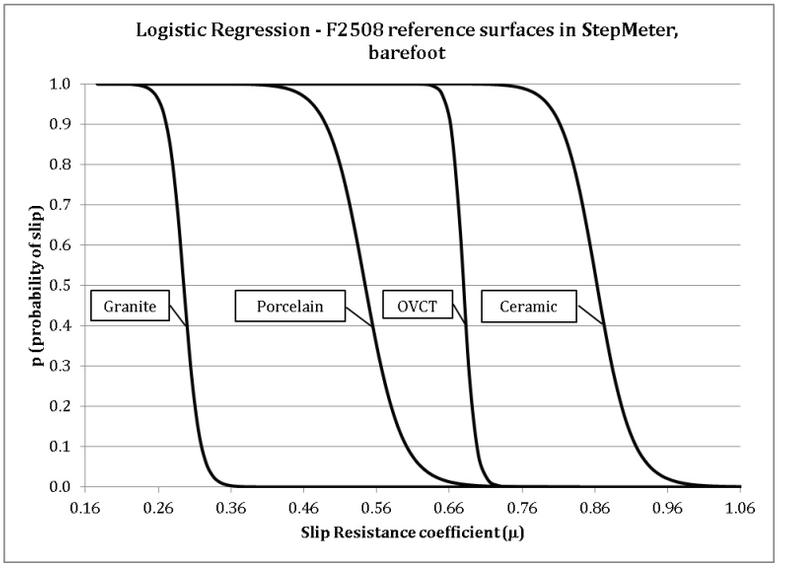

Figure 2: ASTM 2508 StepMeter assessment using logistic regression of a seated barefoot subject.

Redesign: A redesign of the StepMeter was undertaken to achieve two general goals: improving the trial-to-trial repeatability of testing and automating the testing process. To improve the trial-to-trial repeatability of testing, the redesign addressed the following areas: (1) the manner in which the test surface was inclined was re-engineered to allow for greater precision in angular incrementation; (2) the manner in which the test surface was inclined was re-engineered to create a constant "drop" distance; (3) the carriage used to maintain a vertical lower leg was re-engineered to maintain better torsional rigidity; (4) the attachment of the device to the test subject was re-engineered to allow a greater range of test subjects; (5) a system was developed for automatically incrementing the surface inclination between trials; (6) a system was developed to automatically lift the leg to prepare for the next trial; (7) a latch/release mechanism was developed for "dropping" the foot onto the test surface; and, (8) a system to automatically identify and classify a trial as "slip" or "no slip" was developed.

Endpoint: Reliability of a measurement is affected by three factors: (1) the testing apparatus; (2) the tester (the person making the measurements); and, (3) the lability of that which is being measured. It is suspected that this third aspect is paramount for barefoot skin friction; the *in vivo* foot changes with repeated testing, and subject-to-subject differences in the foot, may make ASTM F2508 inapplicable to barefoot slip-resistance characterization. The redesigned StepMeter will allow the collection of a cohort of data on barefoot subjects to characterize the within- and between-subject variation in barefoot pedestrian slip resistance on different surfaces.

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Walkway Safety, Barefoot Tribometry, StepMetern

GENERAL

E1 Difficulties in the Interpretation of Postmortem Concentrations of Synthetic Cannabinoids

Anders Rietz, National Board of Forensic Medicine, Fogdevägen 1, Umeå, Västerbotten 90355, SWEDEN; Gunilla Thelander, BSc, National Board of Forensic Medicine, Dept of Forensic Genetics and Forensic Tox, SE-58758, Linköping, SWEDEN; and Robert Kronstrand, PhD, National Board of Forensic Medicine, Dept of Forensic Toxicology, Artillerigatan 12, Linkoping SE 587 58, SWEDEN*

After attending this presentation, attendees will better understand the increasing use of Synthetic Cannabinoids (SCs), how to evaluate their presence in fatal intoxications, and references of blood concentrations for one substance — MMB-CHMINACA.

This presentation will impact the forensic science community by increasing knowledge regarding how to evaluate the presence of SCs in fatal intoxications by providing results from 49 autopsies categorized to be related to an SC.

SCs were introduced on the drug market in the early 2000s as a "safe and legal" alternative to cannabis and were sold in ready-to-use plastic bags with herbs for smoking. Recently, serious adverse effects, including deaths, from the use of SCs have been reported. Possible explanations for the deaths are inexperienced users, the high potency compared to cannabis, and the sale of pure compounds in large containers. Only very limited information is presented in the literature regarding toxic levels of SCs and most of the different SCs have not previously been reported at all.[1] The goal of this study was to enlighten the toxicology of SCs through analyses of SC-positive cases. The autopsy findings and circumstances in each case were scrutinized and are presented with postmortem femoral blood concentrations of one of the more prevalent SCs, MMB-CHMINACA.

Materials and Methods: Data from medicolegal autopsies in Sweden from 2010 through March 2015 (*N*=approximately 23,100 cases) where SCs were present in blood were reviewed regarding geographical spread, age, sex, cause of death, place of death, and autopsy/toxicology findings. The cases were categorized according to the cause of death by applying a modified version of the strategy proposed by Druid and Holmgren: (1) death due to intoxication of SCs alone (no other drugs detected) or otherwise directly related to intake of SCs; (2) death due to intoxication by SCs *and* other drug(s), and/or alcohol; or, (3) other cause of death not related to the presence of SCs.[2]

Data comparisons were made between levels of MMB-CHMINACA in autopsies and living controls (concentrations detected in suspected drugged drivers).

Results: Forty-nine cases were included of which 92% were male and the median age was 29.7 (range 16 years-59 years). Most decedents were found dead at home. A full autopsy, including histology (90%) and a comprehensive toxicological screening for drugs and medication (100%), was performed, including screening for 50-100 SCs depending on the year. In 41 cases, the cause of death was intoxication and/or related to drug abuse. A total of 24 different SCs were found, with the most prevalent being THJ-018. Preliminary classification resulted in 5 cases in group A, 19 cases in group B, and 25 cases in group C. The forensic pathologist often phrased the death certificate with a low degree of certainty, such as "the death may be caused by…" All but five cases had other drugs present in femoral blood and many had opioids like methadone present, indicating that many victims were experienced drug users. The concentrations of MMB-CHMINACA in femoral blood were comparable to those recorded in living controls.

A Typical Case Report: An 18-year-old man with a multi-substance drug abuse history visited a party, became unresponsive, and was pronounced dead in the emergency room. Toxicological analyses detected nothing but MMB-CHMINACA at a concentration of 0.003 microgram/gram femoral blood. The medicolegal autopsy revealed no other findings that could explain the death.

Discussion and Conclusions: The toxicity of SCs is largely unknown with little data to rely on, implying a risk of misinterpretation of SC intoxications. More results from living controls are needed to enable more reliable judgments regarding toxic and fatal concentrations.

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1. Behonick G., Shanks K.G., Firchau D.J., et al. Four postmortem case reports with quantitative detection of the synthetic cannabinoid. *J Anal Toxicol* 2014;38:559-562.
2. Druid D., Holmgren P. A compilation of fatal and control concentrations of drugs in postmortem femoral blood. *J Forensic Sci* 1997;42:79-87.

E2 Technical Considerations for a Drone-Mounted GoPro® Camera for Crime Scene Measurements

Jacob Martin, Forensic Science Program MS 126, Utah Valley University, 800 W University Parkway, Orem, UT 84058; Annalie Martin, Forensic Science Program MS 126, Utah Valley University, 800 W University Parkway, Orem, UT 84058; and Gary H. Naisbitt, PhD, Utah Valley University, Criminal Justice Dept, MS 286, 800 W University Parkway, Orem, UT 84058*

The goal of this presentation is to assess the feasibility of a drone-mounted GoPro® camera for crime scene documentation.

This presentation will impact the forensic science community by increasing awareness of the benefits and limitations of new technology.

GoPro® cameras have produced new types of dramatic, real-life photography, while drones have introduced low-cost aerial photography. This study asks whether a drone-mounted GoPro® camera is suitable for crime scene measurements. The idea is obvious and simple. The investigator would fly a camera-mounted drone over the crime or accident scene, take the picture, and perform the documentation measurements with photo editor software.

Potential benefits of an aerial photo include: (1) recording the scene before entering it; (2) guarding against accidently moving objects; (3) finding a different perspective that shows the relationship of objects one to another; and, (4) helping to visualize the path the suspect/victim took.

However, there are several technical considerations to be assessed before data obtained in this way can be trusted.

Materials and Methods: A GoPro® HERO4 Black camera equipped with a 32-gigabite micro Secure Digital (SD) memory card mounted with a flexible GoPro® mounting accessory was used to attach the camera to a tripod or drone. A three-foot square, black-and-white target pattern with one-inch squares and converging lines indicating National Television System Committee (NTSC), Phase Alternating Line (PAL), and High-Definition Television (HDTV) resolution was used as a reference scale for visual acuity.

A 1,080p HD video setting gave the best picture resolution. Video was preferred because the two-second delay to the digital display caused real-time uncertainty regarding targeting and orientation to the ground. Single video frames that produced the best overall pictures of the desired target were selected. The file format of the chosen frame was converted for importation into a photo editor, then used to measure distances between objects of evidence, correct visual distortion, and to increase magnification to see detail.

Results: GoPro's® standard issue lens is a fisheye design that bends straight lines. Photo editing software can straighten the lines but distorts the expected square grid, making digital measurements questionable. For an additional cost, GoPro® sells a rectilinear lens that will be evaluated in a subsequent study.

The focal plane of the camera must remain parallel to the surface being photographed. If it is not parallel, angular distortion causes parallel lines to converge at the side of the picture closest to the ground and diverge at the side farthest from the ground. Rocking the drone in flight while taking video is a workaround, but there is not a built-in sensor to identify the frame that is parallel to the ground.

Visual acuity is the ability to see detail. Major contributing factors in digital photographs are: (1) the number of light-sensitive points (pixels); (2) the number of rows and columns in the raster pattern; (3) the distance from the subject; and (4) the intensity of available light.

A video frame is composed of lines. The higher the number of lines per frame and the higher the number of pixels per line, the higher the resolution. HD video uses 1,080 lines per frame with 1,920 pixels per line (1,920 x 1,080) in a 16:9 aspect configuration. Because the number of pixels is constant, visual acuity diminishes with increasing distance and lower light intensity. The ability to clearly see one-inch squares on the target pattern was determined and plotted in Exposure Values (EV) and distance from the target pattern. Additionally, visual acuity also depends on contrast between the evidence and background, automatically set aperture opening and shutter speed, weather conditions, and camera/drone stability.

Discussion: Drones greatly enhance access and maneuverability in a crime scene but have inherent limitations. By knowing the limitations and choosing appropriate equipment, a drone-mounted camera can be a useful tool, but will probably not replace established practices.

Crime Scene Measurements, GoPro®, Unmanned Aircraft

E3 Homicide Injury Quantification: Correlations and Reliability of Injury Severity Scores Applied to Homicide Victims

Fredrik Tamsen, MD, MSc, The Swedish National Board of Forensic Medicine, Rättsmedicinalverket, Box 1024, Uppsala 751 40, SWEDEN; Fia Klötz-Logan, PhD, Uppsala University, Centre for Research and Development, County Council of Gävleborg, Gävle 80188, SWEDEN; and Ingemar Thiblin, PhD, Uppsala University, Rättsmedicinalverkets Rättsmedicinska Avdelning, Box 1024, Uppsala 75140, SWEDEN*

After attending this presentation, attendees will understand the relevance of using injury severity scores in homicide research. Attendees will also understand why injury severity scores that are valid in trauma research are not automatically valid in homicide research. Finally, attendees will be given suggestions of which scores to use.

This presentation will impact the forensic science community by suggesting suitable injury severity scores to be used in homicide research. With an injury severity score, the brutality of the lethal violence can be compared over time, between regions, and between different characteristics of the victim and offender, such as relationship and drug use.

Introduction: An injury severity score is used to summarize a person's injuries with a single number. In trauma research, such scores are used to predict mortality and morbidity on a group level.[1] The scores can be used to assess the effectiveness of trauma care and to compare different regions and time periods. In homicide epidemiology, injury severity scores are rarely used; however, there are interesting questions that could be addressed with an injury score: Has there been a brutalization of violence over time? Are there correlations between the degree of violence and the characteristics of the victims and perpetrators, such as sex, age, drug use, and relationship? This could in turn be one piece of information to use in offender profiling.

A valid injury severity score for homicide victims should capture the overall assessment of the injuries, not just the lethal ones. It should, on a group level, reflect the subjective assessment of brutality. A homicide victim with injuries that most professional assessors would deem as a "brutal killing" or "overkill" should score higher than a victim with a "normal amount" of injuries. Most professional assessors would probably agree that the number of injuries and their individual severities are two key aspects when assessing the overall degree of violence in a homicide victim. The Abbreviated Injury Scale (AIS) is a well-established classification system for injuries, assigning each injury a number from one (minor) to six (maximal). When the AIS classifications of all injuries are summed to create the Sum of AIS (SAIS), both the number of injuries and their individual severities are accounted for. Therefore, it is argued that the SAIS can serve as a gold standard for quantifying the degree of injury in homicide victims.

Method: In a study including 103 homicide victims, the degree of injury was assessed using the SAIS together with five other injury quantification methods: the Injury Severity Score (ISS), the New Injury Severity Score (NISS), the International Classification of Disease Injury Severity Score (ICISS), the Total Number of Injuries (TNI), and the Homicide Injury Scale (HIS).[2] The ISS, NISS, and ICISS are all established in trauma research, while the TNI and HIS were developed for homicide victims. The validities of these methods were evaluated through their Spearman's rank correlations with the SAIS.

The HIS consists of a scale from one to six, in which six is the most severe.[3] The definition of the scale is easily understood and contains both qualitative and quantitative elements. By transforming some of the qualitative elements in the original definition to quantitative ones, the scale was made less ambiguous.

Results: The HIS, ICISS, and TNI showed strong correlations with the SAIS (0.72, -0.61, and 0.81, respectively), while the ISS and NISS showed weak correlations (0.31 and 0.23, respectively).

Discussion: Of the three methods that showed strong correlations with the SAIS, the HIS is by far the quickest to apply. Both the ICISS and TNI can be time-consuming when there are many injuries. The weak correlations of the ISS and NISS are not surprising. These two methods at most only take three injuries into account and even though they are valid in trauma research, they fail to capture the overall injury severity in homicide victims.

Conclusion: The HIS has the combination of good correlation with the SAIS, together with time-efficient assessment. The HIS might therefore be a suitable way to quantify the degree of injury in homicide victims, especially on large case series.

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3. Safarik M.E., Jarvis J.R. Examining attributes of homicides: Toward quantifying qualitative values of injury severity. *Homicide Stud* 2005 9(3), 183-203.

Homicide, Injury Severity Score, Quantification

E4 A Case for Using Mixed Method Research to Investigate the Relationship Between Art and Science in Forensic Facial Reconstruction

Daniel Marion, Jr., PhD, 2452 Gaylord Street, Denver, CO 80205-5630*

After attending this presentation, attendees will be aware of the ill-structured, liminal, superorganic, multistable nature of the facial reconstruction process.

This presentation will impact the forensic science community by opening a methodologically informed conversation within the forensic arts about mix method research and the art/science of the work.

There is an unexamined operational confluence of art and science associated with the working process of developing a forensic facial reconstruction. The commingled art and science of this working process has characteristics of an ill-structured problem.[1,2] Ill-structured problems, as opposed to well-structured problems, require multiple procedures to reach a single correct solution. Social, economic, and political controversial issues are examples of ill-structured problems.

The intent of this presentation is to make a case for the use of mixed qualitative-quantitative research methodologies as an appropriate means to investigate the ill-structured, commingled contributions of art and science in the facial reconstruction process.[3,4] Mixed research methodologies are better suited to represent a balanced account of the contribution of subjective art — image making — and how it flows together with the objective science — anatomy — in the facial reconstruction process. A mixed-methods accounting for the elements of facial reconstruction process throws light on its overlooked ill-structured nature.

The ill-structured aspect of the facial reconstruction problem exhibits the combined qualities of: (1) a superorganism; and, (2) the concept of liminality.[3,5]

A superorganism is a large collective social organism comprised of smaller member organisms that each have specific divisions of labor; these work in concert toward mutual welfare and the larger organism's collective common good. A honey bee hive is an example of a superorganism in which its members (the queen, the workers, and the drones) have individualized labor tasks that collectively ensure the hive will survive. The art/science relationship within the facial reconstruction working process is analogous to a superorganism. Art and the science, as individual organisms, have specific contributions to make toward the outcome of the finished facial image. The balanced input of both art and science are required for a reasonable likeness of a facial reconstruction to be achieved.

Liminality, an anthropological term, is derived from the Latin word for threshold.[3,5] It refers to the feeling of disorientation when passing from one state of being to another state of being. The rite of passage is the best example of this concept. There are two fundamental perspectives from which to understand the working process of a facial reconstruction: the objective scientific aspect and the subjective artistic aspect. A mixed methods investigation into the working process of the art/science relationship of a facial reconstruction will highlight its liminality. A state of disorientation occurs when whichever conceptual orientation toward facial reconstruction one holds changes, as the passage from art to science or science to art is made. The disorientation of liminality also has the conceptual effect of being in the condition of multistability.[4] A scientific example of multistability is the Schroedinger's Cat thought experiment, in which the cat can be considered to be both dead and alive at the same time. A convenient artistic example of multistablity is a Necker Cube, in which all orientations of the Cube are present as the same time.

Treating the facial reconstruction process as a combination of both science and art can result in the benefits of each.

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Facial Reconstruction, Ill-Structured Problems, Liminality

E5 A Unique Case of Death by Misadventure Due to Electrocution Involving a Man and a Cat: The Utility of Electron Microscopy

Elvira Ventura Spagnolo, University of Palermo, Dept of Biotechnology and Legal Medicine, Via Del Vespro n. 129, Palermo 90127, ITALY; Cristina Mondello, BS, University of Messina, Dept of Biomedical Science and of Morphologi, Via Consolare Valeria - Gazzi, Messina 98123, ITALY; Stefania Zerbo, MD, Via Del Vespro, 127, Palermo 90100, ITALY; Antonina Argo, Via Del Vespro 127, Palermo 90100, ITALY; Luigi Cardia, via M. Amari 1, Messina, ITALY; Francesca Giuffrida, Via Enna 1/c, Catania 95128, ITALY; and Giulio Cardia, University of Messina, Dept of Biomedical Science and of Morphologi, Via Consolare Valeria - Gazzi, Messina 98123, ITALY*

After attending this presentation, attendees will understand the importance of a proper methodological approach which takes into consideration the different data available, including the autopsy on an animal, to identify the time, cause, and means of death as well as to reconstruct the crime scene.

This presentation will impact the forensic science community by providing a practical example of the utility that a multidisciplinary approach and cooperation among the different disciplines may have by making good use of the integration of forensic radiological, toxicological, chemical, and anatomo-histological investigations in the identification of the cause of death, especially in those cases with a total lack of certain distinctive elements and/or in the presence of confusing elements, as may happen in cases of death by electrocution.

The diagnosis of electrocution, in addition to being based on circumstantial and investigation data, is essentially based on the discovery of signs of electricity inside the body that, when latent and therefore unidentifiable, may be misleading. In these difficult cases, forensic investigation should use all tools available to the investigator (computed tomography, histology, optics, electronics, and chemical metal detectors) without underestimating any element of the scene investigation.

In this example, the body of a naked man with a widespread brownish color on his skin was found near the metal fence in the garden of his house. Some burnt scraps of material, pieces of jewelry, and other evidence were also found close to the body. The carcass of a cat was discovered some meters away from the body. After the crime scene investigation, the judiciary ordered an autopsy for the purpose of determining the cause, time, and means of death. The medical examiner also ordered an autopsy of the cat.

During the man's autopsy, the presence of second- and third-degree burns on most of his body surface was found, with clear evidence of burned eyebrows, eyelashes, and moustache. No further macroscopic findings were described. Skin and organ fragments as well as the heart were further analyzed. The histological examination of the heart highlighted a plurifocal presence of coagulative necrosis. The skin fragments that were examined revealed an elongated shape of the basal cells perpendicularly oriented compared to the basal lamina, which sometimes exhibited a "tufty" shape with a plurifocal aspect of coagulative necrosis of the superficial and deep dermis. The skin taken from the left hand showed a fragment of skeletal muscles with myofibril contraction bands and myocells undulation, as well as areas of elongation of those cells belonging to the epidermal basal layer, overtopped by micro-bubbles separating the epidermal superficial layers. Small frozen lung sections were fixed in glutaraldehyde at 2% with pH 7.4. Subsequently, samples were dried at critical point and metalized with a gold thickness of 15 angstrom. The scanning electron microscope examination showed the presence of ultramicropores at the cell membrane level of the lung arteries' endothelium.[1,2] Using specific equipment, the metallization evidence was searched and found on the skin.[3] Chemical and instrumental tests performed on the fragments of clothes and other evidence excluded signs of liquids and/or inflammables.

The autopsy on the cat did not show distinctive external signs, but highlighted the spread fragmentation of cardiac muscle fibers and plurivisceral congestion.

The forensic department investigations discovered electrical dispersion starting from an electrical transformer kiosk substation, which on the day the event occurred recorded a short blackout in the pertinent area.

In conclusion, this case highlights the importance of a multidisciplinary approach and the use of second-level techniques in the resolution of individual cases.

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Forensic Sciences, Electrocution, Electron Microscopy

E6 The Value of Outsourcing Selected Cases in a Medical Examiner Population: A Ten-Year Experience

Brandi C. McCleskey, University of Alabama at Birmingham, 619 19th Street, S, Birmingham, AL 35249; Stephanie Reilly, MD, University of Alabama at Birmingham, 619 19th Street, S, Birmingham, AL 35249; and Daniel Atherton, HSB 175J, 619 19th Street, S, Birmingham, AL 35249*

After attending this presentation, attendees will be able to determine the efficacy of a referral arrangement with a university-based autopsy service, establish criteria for a referral system, and evaluate the ability of the system to identify cases for referral.

This presentation will impact the forensic science community by providing an opportunity to improve workflow and decrease workload without compromising quality while generating continued forensic science interest among trainees.

In the United States and other countries, death investigation is often hampered by inadequate staffing, creating a difficult burden for coroner/medical examiner offices. Moreover, in recent years, caseloads have increased drastically, as most jurisdictions have experienced dramatic increases in deaths due to both prescription and illicit drug use. The National Association of Medical Examiners (NAME) recommends that caseloads for Forensic Pathologists (FPs) not exceed 250 autopsies/year. Offices in which FPs perform greater than 325 autopsies/year may have difficulty attaining accreditation or possibly lose accreditation. Since 2006, pathologists at the University of Alabama at Birmingham (UAB) have performed select autopsies for the Alabama Department of Forensic Sciences (ADFS). The purpose of this study is to report on ten years of experience performing autopsies for the ADFS and analyze the efficacy of outsourcing select medical examiner cases to a university-based autopsy service.

Standard forensic protocols and orientation were provided to the university-based pathologists at the initiation of the program. Data for this study includes completed cases from June 2006 through July 10, 2015. For each case, a state FP reviewed the findings of the scene investigator and determined if the case was appropriate for referral. Exclusion criteria included homicides, motor vehicle accidents with pending criminal charges, decomposed or unidentified bodies, and most pediatric cases. All referred cases received full postmortem examination including microscopic examination of select organs; vitreous, blood, and urine were routinely collected on all cases, and toxicology was ordered as appropriate. The referring FP and the state chief medical examiner were available for consult via phone or in person, as needed. A written report with all anatomic and microscopic findings was submitted to the state FP. The final cause and manner of death were determined by the referring state FP after review of the scene investigation, autopsy findings, and toxicological findings.

A total of 414 cases were referred for which cause and manner of death determination were completed: 237 cases were ruled accidental deaths (180 due to drug toxicity, 43 due to trauma, 6 due to drowning, 4 due to burns, and 4 other); 168 cases were due to natural disease with the majority of these being due to cardiovascular disease; 3 cases were ruled suicides; and 6 were ruled undetermined causes and manners of death. Five of the cases had suspicious internal injuries that raised the possibility of foul play. After additional information was obtained, including additional scene investigation or consultation with the referring FP, homicide was ruled out in all these cases. To date, no referred case is pending in the court system.

In conclusion, outsourcing of select forensic cases can be an effective tool to manage workflow without compromising quality. There are many benefits to such a referral arrangement, including helping diminish caseloads of state FPs so they can concentrate on more time-consuming cases such as complex homicides, assuring pathology residents adequate autopsy experience, and providing pathology residents with increased exposure to forensic pathology early in residency, which may increase interest in forensic pathology. Furthermore, a university-based outsourcing arrangement has the potential to provide microscopic examinations and onsite subspecialty consultation for certain cases, postmortem studies that are not always necessarily available to all medical examiners' offices.

Autopsy, Referral, Investigation

E7 The Birth of a National Department for Legal Medicine in the Grand Duchy of Luxemburg

Ulrich S. Preiß, MD, Laboratoire National de Sante, 1, rue Louis Rech, Dudelange L-3555, LUXEMBOURG; Patricia Lambert, MS, Laboratoire National de Santé, 1 rue Louis Rech, Dudelange L-3555, LUXEMBOURG; Sarah Toussaint, Laboratoire National de Santé, 1 rue Louis Rech, Dudelange L-3555, LUXEMBOURG; and Andreas Schuff, PhD, Laboratoire National de Santé, 1 rue Louis Rech, Dudelange L-3555, LUXEMBOURG*

After attending this presentation, attendees will better understand the immediate and gradual challenges encountered by health professionals within the framework of the establishment of a national department for legal medicine.

This presentation will impact the forensic science community by providing insight on the challenges of the creation of a new department of legal medicine.

As a result of its geographical location nearly at the heart of the European Union, with a parliamentary democracy implemental form of government, a population of nearly 550,000 and a surface of 2,586 km^2, a moderate occurrence of violent crime, and a relatively low rate of autopsies, the government of the Grand Duchy of Luxemburg was, in the past, able to outsource its medicolegal services to neighboring countries. In a majority of cases, these services were provided by a legal medicine institute in Germany close to Luxemburg's borders.

In the past, whenever a case arose which required a medicolegal investigation, a German institute was contracted (among others, the Homburg University Hospital, in the proximity of Ramstein/Landstuhl), which then dispatched a forensic pathologist to Luxemburg; however, given the increasing number of cases and the need to increase efficiency, the government of the Grand Duchy decided in 2012 to establish its own legal medicine institute in its own country. This newly created Department for Legal Medicine was thus able to start work in the brand new building of the Laboratoire National de Santé (LNS) in Dudelange (near the French border). Two forensic pathologists working at the institute started on April 1, 2014.

The forensic and clinical toxicology department of the LNS, founded in the 1980s, was also incorporated into the same institute in April 2014. The laboratory's department for forensic genetic identification, which is currently located in Luxemburg city, is scheduled to take up residence in the same building as the Legal Medicine and the Toxicology Departments in 2016.

In addition to geographical difficulties, legal issues had and still have to be resolved (for instance, the necessary revision of laws regarding the statute and transportation of a human corpse, etc.)

In Anglo-Saxon law, a coroner is an investigating agent and is assigned the task of determining the identity of the deceased as well as the cause of death in cases in which the cause of death is questionable or not natural; however, in most European countries, including Luxemburg, the profession of coroner does not exist. In fact, as a rule in Europe, a medical practitioner is called after the discovery of a corpse to proceed with an external postmortem examination onsite and complete a death certificate which states, among other facts, the identity, the manner, the cause, the time, and the location of death. If this medical practitioner's examination shows that the manner of death is or may be unexplained or unnatural, the medical practitioner is obligated to inform the police and the public prosecutor's office in order to open an official investigation/inquest. Thereafter, the deceased undergoes an external and internal postmortem examination. This examination is handled by specially trained medical practitioners, who are equivalent to American forensic pathologists.

Birth of Department, Legal Medicine, Coroner

E8 The Value in Integrating Emergency Management and Forensic Death Investigation at the Harris County Institute of Forensic Sciences

Allison Woody, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; Jason M. Wiersema, PhD, Harris County Institute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054; Phong Nguyen, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; and Roxanne Phatak, MS, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will gain insight into the strategic and operational benefits of integrating emergency management concepts into the management of a forensic death investigative operation. The Harris County Institute of Forensic Sciences recently integrated two previously disparate divisions, Forensic Emergency Management and Forensic Investigations, in the interest of maximizing the efficiency of both functions. This presentation will highlight decreased medicolegal scene response times as one indicator of the overall improvement in death investigation efficiency.

This presentation will impact the forensic science community by illustrating the benefit of incorporating emergency management concepts into death investigation.

The integration of the two divisions allowed for the application of emergency management concepts into the broader goal of forensic death investigation, including: implementation of a manageable span of control and delegation of authority for supervisory forensic investigative staff; revision and development of standard operating procedures within investigations and in collaboration with other divisions, disciplines, and agencies; development of a robust multidisciplinary training program; application of quality assurance and control measures on training and operations; and grant acquisition for necessary equipment to enhance scene response, forensic death investigation, and mass fatality incident management. Within six months of the division integration, tangible benefits of applying these emergency management concepts were observed in decreased scene response times without compromising the quality of scene investigation.

The scene triage protocol, implemented in January 2015, emphasized the scene response prioritization of traffic-related fatalities on major roadways (including freeways and toll roads) over other scene responses, even when it required the diversion of an investigator from another scene. The higher prioritization of these scenes is necessary due to the significant community and roadway impact and safety concerns for first responders and other motorists. The protocol and subsequent training was developed in coordination with law enforcement and local and state transportation agencies, allowing for the reconciliation of each agency's requirements and operations prior to implementation.

Medicolegal scene response times from 2014 and 2015 were compared as one measure of the impact of the protocol implementation. Since the change in protocol, motor vehicle accident deaths scene response times decreased by 25.9%. The decrease in response time was an anticipated result of the protocol implementation; however, the data comparison also revealed significant decreases in response times to all other case types. Natural deaths scene response times decreased by 12.8%, suicide deaths scene response times decreased by 5.8%, and homicide deaths scene response times decreased by 12%. The overall decrease in scene response times is directly attributable to the implementation of the integrated approach.

Death Investigation, Emergency Management, Medical Examiner

E9 Stress Responses of Crime Scene Investigators When Responding to Traumatic Death Events

Jalika Rivera Waugh, PhD, St. Petersburg Police Department, 1300 1st Avenue, N, St. Petersburg, FL 33710*

After attending this presentation, attendees will: (1) learn about the difference between traumatic event-related stress and occupational stress; (2) learn about traumatic events as they relate to crime scene environments; and, (3) learn about survey instruments used to gather data.

This presentation will impact the forensic science community by providing insight into the difficult responsibilities during a crime scene investigation and the potential stress investigators usually encounter.

The research literature has identified that first responders such as law enforcement officers, firefighters, and emergency medical personnel often experience extreme levels of stress. Aside from jurisdictions where Crime Scene Investigators (CSIs) are sworn law enforcement officers, such as the New York Police Department, the Connecticut State Police, or the Massachusetts State Police, most police departments and sheriff's offices employ civilian personnel to respond to and process traumatic death events. Their duties may include collecting and preserving physical evidence from the deceased and from the entire crime scene, as well as performing a variety of routine and complex technical criminal investigation work such as determining the usability of latent fingerprints, trace evidence, and biological evidence, by taking photographs and testifying effectively in court. As such, CSIs, who may have either a civilian support staff or a law enforcement officer role, also respond to critical incidents (such as traumatic death events) and thus are also likely to experience similar levels of traumatic stress.

After a review of the research literature, it was found that there were no studies that focused on civilian support staff that process traumatic death events. As such, a non-experimental survey research methodology, using the Impact of Event Scale-Revised (IES-R), measured participants' perceived levels of event-related stress. Data were collected from a sample of crime scene investigators who are members of two professional forensic organizations (one national and one based in Florida). The research indicated no correlation existed between levels of self-reported measures of stress and the amount of traumatic death events experienced. Additionally, the research indicated that no correlation existed between variables of gender, age, and military combat experience and levels of self-reported measures of stress. It was observed in the raw data that outliers from the study reported high measures of stress across different variables. Participants were asked (prior to taking the survey) if they would provide additional information in future qualitative research studies regarding specific stressors when responding to traumatic crime scenes. A discussion will be provided regarding future research in which participants will be offered an opportunity to expound upon their quantitative responses. In this scenario, participants would be given the opportunity to expand upon the closed-ended questions not addressed in the IES-R.

Traumatic Stress, Crime Scene, Quantitative Methodology

E10 Deaths in Silence: The Role of Prison Surveillance in Suicides

Isabella Aquila, MD, Viale Europa, località Germaneto, Policlinico Universitario, S Venuta-Medicina Legale, Catanzaro 88100, ITALY; Silvia Boca*, Viale Europa, Catanzaro, ITALY; Ciro Di Nunzio, MFS, PhD, Magna Graecia University, Viale Europa, Germaneto, Legal Medicine, Catanzaro 88100, ITALY; Salvatore Savastano, Viale Europa, 88100 Germaneto, Catanzaro 88100, ITALY; Francesca Pepe, MD, Viale Europa, località Germaneto, Catanzaro 88100, ITALY; Santo Gratteri, MD, Viale Europa, Germaneto, Catanzaro 88100, ITALY; and Pietrantonio Ricci*, Viale Europa-Località Germaneto, Catanzaro, ITALY*

After attending this presentation, attendees will be able to describe the impact of surveillance systems in prison in order to reduce the risk of suicide.

This presentation will impact the forensic science community by demonstrating the importance of surveillance systems especially when the medical history of prisoners' psychiatric disorders or past self-harm are detected.

Introduction: In Italian prisons, inmates commit suicide at a rate 19 times greater than non-incarcerated people and they often do so in institutions where the living conditions are worse, such as in particularly dilapidated structures with few activities and a minimal presence of volunteers. In some cases, people who have committed suicide were suffering from disabling diseases and hospitalized in penitentiary clinical centers. The causes of death in prisons can occur for natural reasons, as a result of pre-existing illnesses, from violent acts related to self harm, or due to murder between inmates, usually as a result of fights in prison. In particular, there are a greater number of suicides. Psychiatric disorders increase the risk of suicides and they are usually higher among male than female prisons. Moreover, the most widely used method of suicide is represented by hanging, followed by choking, cutting veins, intoxication, and drug overdoses. In Calabria, Italy, deaths of those in detention are highly related to suicide. In Catanzaro, Italy, the percentage of deaths in custody due to self harm is in line with those overall. The frequency with which these crimes occur with people previously experiencing self harm is high. Suicides also increased among foreign prisoners. When there is a death in custody, the inspection is complicated. In particular, the narrow and small space in which it occurs may cause errors of judgment from the forensic pathologist.

Case Report: Calabria experiences many cases of suicide in prison. Reported is the case of a Tunisian boy, detained for theft, who in December 2013 was found dead in his cell. The corpse appeared suspended by the neck by a sheet tied to the cell bars. The victim was kneeling with his head tilted sideways. A forensic pathologist was appointed to clarify the manner of death and, in particular, the possible intervention of other factors which would have determined the death. The corpse appeared cyanotic with subungual and lip cyanosis. Hypostases were plentiful and red as in deaths by asphyxia. Evaluations on the suspension mechanism employed were performed. Measurements of the point where the sheet was bound and the knees of the corpse were taken and the cell was inspected. There were numerous cut scars over the boy's body from previous suicide attempts. The medical records of the prisoner was examined and also reveled numerous suicide attempts. The boy suffered from severe depression and his disorder was treated with antidepressant medications. Depositions from the that night's prison personnel were collected. The autopsy showed evidence of hanging and the hypothesis of suicide by hanging was confirmed. From this forensic case, it emerged that the detainee had not been carefully monitored by prison workers and medical personnel in spite of suicide attempts in the previous months and especially of his serious psychiatric disorder.

Conclusions: The suicide risk in prisons is very high. Surveillance systems to date have not reduced the number of deaths. Surveillance systems in prison should: increase medical checks; generate activities of social integration among prisoners through specific programs; and assess the risk of suicide for every prisoner by inserting a board-qualified psychosocial professional to evaluate the anamnesis and pathologies of detainees. It is hoped that all countries can implement adequate surveillance systems, especially when the medical history of prisoners, psychiatric disorders, or past self harm are detected, in order to respect human rights and avoid the silence in the community regarding deaths in prison.

Surveillance, Hanging, Prison

E11 Survivability of Explosive Residue on Improvised Explosive Device (IED) Components Subjected to an Underwater Detonation

David J. Prasek, MFS, 9702 Evening Bird Lane, Laurel, MD 20723; Ronald L. Kelly, BS, FBI Laboratory/TEDAC, 2501 Investigation Parkway, Rm 4210, Quantico, VA 22135; Ismail M. Sebetan, MD, PhD*, National University, Forensic Sciences Program, 11255 N Torrey Pines Road, La Jolla, CA 92037-1011; and Paul Stein, PhD*, National University, Forensic Science Program, 11255 N Torrey Pines Road, La Jolla, CA 92037*

After attending this presentation, attendees will better understand the viability of explosive residue after a detonation in the water column and how the amount of time in the water may degrade the ability to detect explosive residue as well how different materials will adsorb different amounts of explosive residue.

This presentation will impact the forensic science community by assisting attendees in gaining a better understanding of the effects from the maritime environment and will help improve the protocols supporting the collection of post-blast residue.

This study was conducted to better understand the persistence of explosive residue after a detonation in the water column. Additionally, the effect of the elapsed time in water on the ability to detect explosive residue as well how different materials will adsorb different amounts of explosive residue will be demonstrated. The intent of this study is to evaluate current operating procedures to ensure investigators have the most current information available to them concerning best practices in evidence collection and preservation, as well as to maximize their efforts during an often limited timeframe of access to a post-blast scene.

This research is based on the premise that explosive residues will survive in a maritime environment. This was an empirical study with assistance provided by the Federal Bureau of Investigation (FBI), the Bureau of Alcohol, Tobacco, Firearms, and Explosives (ATF), and Naval Surface Warfare Centers (NSWC). IEDs were detonated in an explosive test pond, then allowed to remain submerged in fresh water for specific periods of time. The goal was to analyze the post-blast debris to ascertain if submersion in water had any effect on the ability to recover and identify post-blast residues and, if so, to identify trends showing any degradation of recoverable residue based upon length of time submerged.

The FBI and ATF laboratories played a significant role in the analysis and identification of the explosives using both gas chromatography/mass spectrometry and liquid chromatography/mass spectrometry. Explosive residue was identified on all of the devices that were detonated. Explosive residue could be recovered from various witness materials tested; however, this testing was unable to establish a relationship between degradation of explosive residue versus time submerged.

This research illustrated that it was possible to identify explosive residue recovered from various types of witness material exposed to a detonation in the maritime environment, with the ideal time to collect residue being within one hour of detonation. Identification of explosive residue was also possible after 120 hours of water submersion after detonation, but the detectable amount of residue was greatly reduced. This information will be useful to investigators who are tasked with responding to maritime explosive incidents by establishing a timeline for evidence collection that would still result in a reasonable probability of successful recovery of explosive residue.

Underwater CSI, Maritime IED, Post-Blast

E12 The Lip Prints Morphological Profile in a Brazilian Population: A Prospective Study

Antonio A. Antunes, PhD, Rua Cardeal Arcoverde, 267, Graças, Recife, Pernambuco, BRAZIL; Raylane F. Albuquerque, Faculty of Dentistry, University of Pernambuco, Av. General Newton Cavalcanti, s/n, Camaragibe, BRAZIL; Patricia S. Trigueiro, MSc, Faculty of Dentistry, University of Pernambuco, Av. General Newton Cavalcanti, s/n, Camaragibe, BRAZIL; Evelyne P. Soriano, PhD, Faculty of Dentistry, University of Pernambuco, Av. General Newton Cavalcanti, s/n, Camaragibe, BRAZIL; Marcus Vitor D. Carvalho, PhD, Faculty of Dentistry, University of Pernambuco, Av. General Newton Cavalcanti, s/n, Camaragibe, BRAZIL; Reginaldo I.C. Campello, PhD, Faculty of Dentistry, University of Pernambuco, Av. General Newton Cavalcanti, s/n, Camaragibe, BRAZIL; and Gabriela G. Porto, PhD, Faculty of Dentistry, University of Pernambuco, Av. General Newton Cavalcanti, s/n, Camaragib, BRAZIL*

After attending this presentation, attendees will better understand the morphological patterns of lip prints in a Brazilian population through the application of specific classifications and will understand which differences are presented if compared with findings of pertinent literature.

This presentation will impact the forensic science community by providing results on the lip prints' morphological profile of a Brazilian population sample. These records are important to provide data in the human identification process, especially in crime scenes.

Human identification constitutes an important step in civil and criminal cases. Dental characteristics, dermatoglyphics, and DNA comparisons are commonly described as systematically used techniques for a rapid and secure identification process; however, in specific conditions in a crime scene or in the absence of experienced staff, some of these techniques may not be available. An increasing interest in alternative and reliable methods of human identification can be found in some studies.[1,2] In order to overcome these limitations, the palatal rugoscopy, cranial measurements in the mastoid process of occipital bone, as well as the clavicle, humerus, radius, and ulna have been cited. Among these techniques is cheiloscopy. Similar to fingerprints and palatal roughness, the labial grooves are permanent and unchangeable. According to some authors, it is possible to identify lip patterns from the sixth week of intrauterine life.[3] From that point on, the lip patterns rarely change, resisting many aggressive agents, such as herpetic lesions.[4] Lip prints found at a crime scene can be the basis for conclusions and can characterize a particular event, such as the number of individuals involved, their gender, habits, occupational aspects, and pathological changes on the lips, as individual information.[5] The lip prints, as well as fingerprints, have been used as genetic markers in several congenital disorders.[6] To date, lips' morphological records of numerous populations have been recorded and published; however, cheiloscopic data of the Brazilian population are scarce in the literature.

To perform this prospective study, individuals were randomly selected, among them patients, students, and staff of the Faculty of Dentistry/University of Pernambuco (FOP/UPE), Brazil. Records with data such as gender, age, Body Mass Index (BMI), ethnicity, and characteristics of the labial commissures were collected. Lip prints were collected in a specific sheet through standardized dark lipstick and lip measurements were taken with a digital caliper. Data were compiled in a database and later analyzed according to Suzuki and Tsuchihashi's and Renaud's classifications. For analysis of labial commissures, individuals were positioned with the Frankfurt plane parallel to the ground and loosened lips. Then, a line parallel to the ground was drawn with the aid of a dental strip. Labial commissures were classified into three types: horizontal (the corners arranged perpendicular to the line drawn in the middle lip tangent line to lip tuber); high (commissures arranged above said row); and lowered (the corners arranged below the aforementioned reference). For the evaluation of results, descriptive statistics such as mean, standard deviation, and percentages were used.

A total of 650 individuals were included in the present study. According to the population distribution as numerical values and percentages, the Suzuki and Tsuchihashi's classification showed that generally the predominant pattern was type I — lips with complete vertical grooves that were superior in most quarters, especially in the first subquadrant in the lower right and left quadrants, reaching values greater than 50%. This was followed by type II — lips with branched grooves, reaching values close to 50% in subquadrants four of the two lower quadrants. Regarding Renaud's classification, it can be seen as a predilection for type B — incomplete vertical grooves with values always above 30%, followed by type A — complete vertical grooves. Some specific types have significant values, but only in some of the sextants, such as type F — incomplete branched grooves, which represents 20% of the sample to the left upper sextant, which may not represent the whole sample.

With the data analysis, it was possible to identify the morphological patterns and their correlations with other studied data, providing important tools in helping the human identification process in civil and criminal areas. The records of this information are of paramount importance in order to obtain reference standards and to create a database, making comparisons in future cases that require human identification possible.

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Lip Prints, Human Identification, Anthropometry

E13 Applications of Plant Sciences to Forensic Science

Jane H. Bock, PhD, 5224 Lighthouse Point Court, Loveland, CO 80537-7916; and David O. Norris, PhD*, 1860 Elder Avenue, Boulder, CO 80304*

The goal of this presentation is to show how students and professionals in the forensic field can apply an under-used form of evidence in their work. This message and work is especially relevant to those who participate in homicide investigations.

This presentation will impact the forensic science community by showing illustrations explaining how evidence from plant science can be utilized in many kinds of homicide investigations, including crime scene evidence, elaboration of autopsy results, and how ecological considerations can be highly significant.

The United States National Academy of Sciences (USNAS) 2009 Report, Strengthening Forensic Science in the United States: A Path Forward, raised serious questions and cautions about the validity and overall quality of forensic science. The American Academy of forensic Sciences (AAFS) has strongly responded to these concerns and is a leader in helping to form national policy addressing these problems. Not addressed by the USNAS are the contributions and values of lesser-known but well-tested forensic tools. This presentation documents the strengths of a generally under-valued but potentially highly valuable aspect of forensic science — forensic plant science. At present, these techniques have not received enough attention, even though they often are both simple and inexpensive compared with current technical lines of evidence. It is believed this lack of use is due primarily to lack of knowledge as to how such evidence can be of use. This presentation illustrates how botanical evidence has multidimensional applications in forensic investigations. Evidence such as that illustrated in this presentation from the plant sciences is readily passed by *Frye* and/or *Daubert* tests.

The application of three aspects of plant science are described here: (1) plant anatomy (microscopic examination of plant cells, tissues, and organs); (2) plant taxonomy (identification of plant species using morphological characters); and, (3) plant ecology (relationships among plant species and their environments). All three contribute in many ways to forensic science. Plant anatomy has proved useful in determining time of death, place of last meal, and people, including suspects who were associated with the victim. Both plant anatomy and plant ecology have been used to connect suspects to particular crime locations, to suspects, to vehicles, to clothing, and to other evidence associated with the crime. Plant taxonomy can be used to identify poisonous plants and plants associated with illegal drugs, to establish links to the crime site, and to places where vehicles and other materials associated with the suspect or the victim have been. Furthermore, evidence using plant anatomy and plant ecology often are dependent upon the proper scientific identification of plant species. Algae, fungi, bryophytes, ferns, conifers, and flowering plants can all be utilized forensically. The roles played by plants in determination of time of death and connecting victims and suspects to crime scenes are emphasized.

This presentation illustrates how these various aspects of botany have been applied to homicide cases in many jurisdictions.

Plant Science, Plant Anatomy and Taxonomy, Plant Ecology

E14 Evaluation of Drug Intoxication Cases and Medicolegal Reports

Kenan Kaya, Çukurova University, Faculty of Medicine, Dept of Forensic Medicine, Adana, Saricam 01330, TURKEY; Mete K. Gulmen, PhD, MD, Cukurova University, School of Medicine, Dept of Forensic Medicine, Adana 01330, TURKEY; Derya Kaya, Cukurova University Medicine Faculty, Pharmacology Department, Adana 01330, TURKEY; Ahmet Hilal, MD, Çukurova Üniversity, School of Medicine, Forensic Medicine Dept, Adana, 01330, TURKEY; and Necmi Cekin, MD, Cukurova University School of Medicine, Dept of Forensic Medicine, Balcali, Adana 01330, TURKEY*

After attending this presentation, attendees will better understand the measures for medicolegal expertise and reports within the obtained data in drug intoxication cases.

This presentation will impact the forensic science community by presenting demographic data of Adana, Turkey, and by examining the problems in medicolegal expertise and reports related to drug intoxications.

Elements that cause impairment in the biological systems and vital functions are referred to as toxic substances; the various damages they cause in the body are known as intoxication.

A clinician should always suspect intoxication in cases that have multiple organ failures with an unknown cause. Only 0.8%-5% of all cases admitted to the emergency services are diagnosed as intoxication; a significant number of those cases are hospitalized and followed in Intensive Care Units (ICUs).[1]

Many types of chemical substances can be recognized as responsible agents in those intoxication cases, such as therapeutic drugs, pesticides, food, fungi, corrosive substances, insecticides, narcotic drugs, stimulants, volatile substances, and war gases.[2] Therapeutic drugs are the most common cause at all ages. The manner of death in most of the adult drug intoxication cases is suicide; however, in childhood cases, this is generally accidental.[3]

Toxic chemicals may be ingested, inhaled, or absorbed through skin/mucosa from parental and/or rectal applications.[4] Antidepressants are the most common therapeutic drugs that cause intoxication. Patients on antidepressants primarily attempt suicide with their own medications, showing a higher ratio of doing so than others. Of patients with previous psychiatric problems, 50% attempt suicide; 95% of all poisoning cases are suicide attempts and most are women.[5] Antipsychotics, anticonvulsants, antidepressants, stimulants drugs, cardiovascular drugs, and antihistamines are the most common drugs in fatal cases. According to reports from the United States Poison Control Center in 2002, more than two million cases of poisonings had been observed and 51.6% of the cases were children under the age of six years.[6]

Ninety-eight therapeutic drug intoxication cases applied to the Forensic Medicine Clinic between the years 2013-2015. All of the cases were examined and evaluated by the clinical forensic medical examiners and were analyzed retrospectively. Sixty-six of all the cases (67.3%) were women. Thirty-two of the cases (32.6%) were between the ages of 21 years and 30 years old; the youngest was two years old while the oldest was 52 years of age. Sixty-four cases (65.3%) had suicidal attempts. Most of the childhood cases were accidentally exposed to therapeutic drugs. Some cases had received overdoses during clinical therapy. Thirty-two cases of all the suicides (50%) used only one kind of therapeutic drug while 19 patients (29.7%) used more than one medication concomitantly. In 13 of the cases (20.3%), the drugs that had been used remained unknown. Seventy-five percent of all suicidal cases were women. Those cases mainly used antidepressant drugs as the chemical agent while analgesics, non-steroid anti-inflammatory drugs, antibiotics, muscle relaxers, and antipsychotic drugs followed respectively. In 20 of the cases (20.4%), life-threatening pathological systemic conditions were reported.

This study revealed significant demographic data which led to a warning and alert for further precautions. Therapeutic drug intoxications are one of the major serious causes of admission to emergency services. Unlike other conditions, those cases recover much better with appropriate treatment and follow up.

Forensic clinical examinations are important in daily practices and may assist many clinicians as well as emergency service and ICU caretakers. Thus, a clinical forensic examiner and expert should design a detailed report to alert and inform clinicians as well.

This study will discuss the clinical forensic medicine applications, examinations, and reporting in a standard base so all medical doctors may improve their services through better understanding.

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Drug Intoxications, Clinical Forensic Medicine, Medicolegal Expertise

E15 Investigation of Human Skeletal Tissue Using Raman Spectroscopy (RS) and Surface-Enhanced Raman Spectroscopy (SERS) for Forensic Applications

Kristin K. Cooke, BS, 250 Pincushion Lane, Apt A4, Cullowhee, NC 28723; and David D. Evanoff, Jr., PhD, Western Carolina University, 231 Natural Sciences Bldg, 111 Memorial Drive, Cullowhee, NC 28723*

After attending this presentation, attendees will understand the main principles behind RS and surface enhancement, characteristic spectra of different skeletal tissue and DNA components, examples of when this pre-extraction screening technique would be useful, and the overall effectiveness of the proposed technique.

This presentation will impact the forensic science community by discussing how sequencing DNA from human bones is generally a very expensive and time-consuming process but, by implementing this pre-extraction screening technique for skeletal DNA, viability could be highly useful in avoiding the sequencing of any degraded, non-viable bone DNA samples.

Detection of DNA in various forms is an essential and oftentimes delicate process that plays a key role in everything from forensic science for crime scene information to diagnostic screening in clinical medicine and other biological sciences applications. In forensic cases dealing with human remains, bones are sometimes the only accessible source of DNA. As such, the extraction of DNA from bone tissue is a widely studied area in forensic science. Unfortunately, there is no standard technique to qualitatively assess the likelihood of obtaining a useable amount of high-quality DNA for sequencing. The purpose of this research is to evaluate the utility of RS or SERS as useful diagnostic tools for determining whether a bone sample contains viable DNA for extraction and sequencing.

Raman scattering is a type of vibrational spectroscopy that can identify functional groups of biologically relevant molecules, allowing each molecule to have a fairly unique "spectral fingerprint." Raman scattering is a very rare event that is difficult to measure in low concentrations; however, Raman signal can be significantly *enhanced*, by placing the analyte on/near a nanostructured *surface* of a noble metal, thus *surface-enhanced* RS. RS has gained considerable interest in recent years for the detection and identification of forensically relevant materials such as human bodily fluids, explosives, and illicit drugs.

Previous research has shown that DNA nucleobases can be observed by SERS and that RS has been used to evaluate alterations to bone composition associated with aging, disease, or injury by distinguishing the mineral and matrix markers from other components of a Raman spectrum of skeletal tissue. Individual Raman and SERS spectra of all bone and DNA components will be presented, as well as investigations of the development of a standard SERS procedure to detect DNA in a bone sample and the effect of environmental conditions on sample spectral markers.

SERS, Bone, DNA

E16 John David Brown Brought to Justice 20 Years Later — A Multidisciplinary Approach to a Cold Case Homicide Investigation

Donald Hayden, MFS, 292 Harbour Lane, Richmond Hill, GA 31324; and Steven Geniuk, MS*, 108 S Johnson Street, Bldg 31022, Fort Huachuca, AZ 85613*

After attending this presentation, attendees will better understand the many facets of cold case skeletal recovery sites. Attendees will be introduced to the many different forensic disciplines used in conducting an excavation of a 20-year-old body disposal site.

This presentation will impact the forensic science community by demonstrating the different disciplines needed to accomplish a cold case homicide skeletal recovery. This presentation will also demonstrate a unique method of engaging young forensic scientists in real world criminal investigation.

Synopsis: In 1985, Mary Ellen Nobles, then age 26, of Bolivar, MO, went missing. The investigation went cold until mid-2005. In mid-2005, the family of Ms. Nobles reported that Mr. John David Brown, who was confined in the Missouri Department of Corrections on an unrelated murder charge, had corresponded with the family and admitted he murdered Ms. Nobles in 1985 and disposed of her body in a wooded area of Pulaski County, MO.

A year-long investigation occurred. Law enforcement interviewed Brown, who provided additional details about the murder and disposal site. Multiple searches using cadaver dogs, aerial overflights, and law enforcement on foot eventually developed a probable body disposal site. The site was a pond overgrown with 20 years of vegetation.

In the spring of 2006, an excavation of the site was planned in concert with several forensic disciplines. First, as the potential remains would be skeletal, a forensic anthropologist and the University of Missouri Human Remains Identification Laboratory was engaged. Second, as the site was in a the Mark Twain National Forest, agents from the United States Forestry Division and the Missouri State Department of Natural Resources were engaged to assist. Next, coordination was established with a local contractor for excavation equipment and the local rural fire department to provide assistance.

In an attempt to further ignite interest in the forensic science field, forensic science and forensic anthropology college students, supervised by their professors, were engaged for the recovery.

On May 22, 2006, a two-day site recovery was accomplished. A total of 18 pieces of skull, including a full mandible, were recovered over the course of two days. Forensic anthropology students, supervised by their professor, made initial determinations as to human or animal remains.

Following the recovery efforts, the services of a local forensic odontologist were engaged to obtain postmortem dental X-rays. Fortunately, pursuant to a court order, antemortem dental records were obtained. The antemortem records had been retained by the dentist because, in his words, "You never know."

Dr. Daniel Wescott, a member of the Anthropology section of the American Academy of Forensic Sciences, and at that time the chair of The University of Missouri Human Identification Laboratory, made a positive identification of Ms. Mary Nobles. In an abundance of caution on the part of the prosecutor, mitochondrial DNA was obtained and a comparison was made confirming Dr. Wescott's opinion.

On January 23, 2008, John Brown entered a plea of guilty to a charge of first degree murder for the death of Mary Nobles. Brown was sentenced to life in prison without the possibility of parole.

This presentation will detail the investigation, the interaction with multiple forensic disciplines (crime scene examination, skeletal recovery, forensic anthropology, forensic odontology, and DNA) and the engagement of young forensic scientists, who used this case as a springboard to their careers. Some of the outside-the-box investigative methods and non-traditional coordination will also be discussed.

Cold Case, Skeletal Remains, Forensic Odontology

E17 A Continuing Need — Certification of Medicolegal Death Investigation Personnel

Julie A. Howe, MBA, Saint Louis University, Franklin, Jefferson & St Charles MEO, College of Health Sciences, 3084, St. Louis, MO 63104-1028; and Steven C. Clark, PhD*, Occupational Research and Assessment, 124 Elm Street, Big Rapids, MI 49307*

After attending this presentation, attendees will be able to identify the various job titles used by medicolegal agencies (medical examiner, coroner, justice of the peace) nationally to describe the employment of individuals to perform medicolegal death investigation as well as the various educational methods available to individuals seeking employment as medicolegal death investigators.

This presentation will impact the forensic science community by showing how each participant in the investigation of death, either directly as scene investigators or indirectly as forensic consultants and educators, should understand the career tracks and educational methods used by medicolegal death investigators.

Medicolegal jurisdictions vary widely across the United States and include medical examiner (county, state or district), coroner (appointed or elected), justice of the peace, and sheriff-coroner systems. Each agency has some level of statutory authority to investigate multiple types of sudden and unexpected death. The educational preparation and certification requirements of the individuals responsible for performing these medicolegal investigations also varies. Many systems are fraught with inconsistent practices associated with leadership changes (i.e., elections) and budget restrictions and are often underfunded and understaffed.[1]

To better understand the educational variability that exists in the field of medicolegal death investigation, a cursory literature review was performed to identify common "job titles" used to describe employment positions held by individuals who work for or with medicolegal agencies. The literature review produced a list of 38 job titles for forensic positions that participate in overall medicolegal investigation of death. A survey was deployed to members of the three organizations identified as the primary employers of medicolegal death investigators in the United States: the American Board of Medicolegal Death Investigators (ABMDI), the International Association of Coroners and Medical Examiners (IACME), and the National Association of Medical Examiners (NAME). The survey was designed to identify the educational requirements possessed by individuals holding job titles associated with medicolegal death investigation. The results appeared to indicate that medicolegal death investigator positions encompass numerous duties, requiring skills associated with various other job titles including office administrator, data analyst, crime scene investigator, evidence technician, photographer, social worker, counselor, and more.

A United States Department of Justice (USDOJ) expert panel on medicolegal death investigation concluded that death investigators must be educated and trained to properly determine the scope and extent of the death investigation and ensure quality for each investigation.[1] The medicolegal investigation differs from the criminal investigation and its results impact both public health and public safety, therefore emphasizing the need for standardized certification of medicolegal personnel. The multitude of job titles suggests a lack of standardized academic preparation for employment as a medicolegal death investigator. In addition, the education level of medicolegal death investigations nationally ranges from a high school diploma (coupled with various on-the-job training activates) to post-graduate degrees in medicine, anthropology, dentistry, and the law.

Numerous undergraduate and graduate forensic science programs exist across the country, although there are few programs specifically designed for medicolegal death investigation. The training courses available in forensic science range from basic on-the-job training (job shadowing) offered by hiring agencies to specialized training to perform specific investigative tasks (i.e., bloodstain pattern analysis, trace, hair or fiber analysis; entomology, etc.). Training is delivered through various methods including traditional classroom, laboratory, internships, mentorships, and hybrid online courses, which may include hands-on training and field experience. Training is offered by colleges, universities, consultants, medicolegal agencies, and professional organizations, but there is an absence of established criteria for content quality and training outcomes. Content delivery is variable, dependent upon the experience and competency of the trainers who are typically non-educators.

Certification ensures that an individual has demonstrated a recognized level of proficiency in the standards associated with the job title and establishes a basic standard of knowledge for a profession. An independent certification body verifies that an individual has achieved a recognized level of proficiency regardless of the educational methodology, agency, or organization offering the training. Certification can provide direction for education providers and instill confidence in hiring agencies who seek individuals that have verified prerequisite job knowledge and established professional knowledge including adherence to a code of ethics and continuing education.

There are numerous obstacles to ensuring that medicolegal death investigators are properly educated, trained, and certified. Funding is probably the largest obstacle, especially for small, rural medicolegal offices. Federal or state funding to assist in defraying costs associated with certification and training through existing or new grant programs would be advantageous.

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1. Scientific Working Group on Medicolegal Death Investigation (SWGMDI) Report and Recommendation for Certification of Medicolegal Investigative Personnel. December 2013. http://swgmdi.org/images/ACET3.PRC10.RecommendationCertificationMDIPersonnel.Published.6.5.14.pdf. Accessed 06.30.15.

Certification, Training, Medicolegal Agencies

E18 Testing the Use of Pigs as Human Proxies in Decomposition Studies

Melissa A. Connor, PhD, Colorado Mesa University, 406 Lowell Heiny Hall, 1100 N Avenue, Grand Junction, CO 81501-3122*

After attending this presentation, attendees will better understand the use of proxies in decomposition studies and factors that may or may not make an animal a good proxy.

This presentation will impact the forensic science community by providing information relevant to decomposition studies that use proxies rather than human remains.

The present study compares decomposition in 19 pig carcasses and 23 human remains. One previous study used five each of pigs, humans, and rabbits and concluded the rabbits differed from the pigs and humans.[1] In the present study, the pigs were euthanized by Gunshot Wound (GSW) to the head. Human cause of death included cancer, lupus, heart attack, GSW to the head, and blunt force trauma caused by a fall. The experiment was conducted at the Forensic Investigation Research Station (FIRS) in western Colorado. The climate is arid, generally receiving less than eight inches of rain a year.

The remains were placed in the facility at different times between September 2012 and February 2015. Environmental data were collected from a HOBO® weather station placed among the remains. Accumulated Degree Days (ADD) were calculated for each day that the Total Body Score (TBS) was recorded.[2] This resulted in 1,381 data points for the human subjects and 1,091 data points for the porcine subjects. Maximum ADD for the first specimen placed in 2012 was 11,269. Preliminary analysis shows both the mean and the median of the ADD at each TBS point is consistently lower in the human sample, although generally within two standard deviations. The samples were closest during early decomposition (TBS 3-16), but the gap grew as TBS increased.

In early decomposition, pigs and humans showed similar patterns of decomposition; however, in almost 60% of the pig specimens, the intestines ruptured through the abdomen during the bloat phase. This occurred in none of the human specimens. Both species plateaued between TBS 21 and 24 for a significant period of time. The humans stayed in moist decomposition for a longer period of time; however, the pigs were animals of a healthy weight and more than half the human sample was overweight or obese. Body fat does impact decomposition, hindering dissipation of heat and providing liquid for bacterial growth.[3] The cause of death in the humans included cancers and injuries. Antemortem infections accelerate putrefaction and wounds provide additional places for insect oviposition.[4,5] In FIRS' arid climate, most remains mummify. The first pig laid out, in September 2012, has a TBS of 24 at this writing. As of this writing, only pigs had progressed past a TBS of 30. The two humans who progressed past a TBS of 24 were very ill at the time of death (lupus and cancer) and decomposed relatively quickly.

Non-human proxies do provide a more homogenous sample, allowing isolation of individual variables. Human samples tend to be more variable, particularly in body fat and cause of death, both of which impact the pattern of decomposition. Pigs may be useful in studying general trends, but they are not a substitute for human subjects. Above all, reliance on a relatively homogenous proxy sample may make researchers overconfident in their ability to predict the timing and patterns of decomposition.

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2. Megyesi M., Nawrocki S.P., Haskell N.H. Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. *J of Forensic Sci* 2005;5 (3): 618- 626.
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Decomposition, Taphonomy, Animal Models

E19 An Unusual Case of Complex Suicide by Nail Gun, Carbon Monoxide, and Ethanol

Erick P. Bryant, MFS, Colorado Bureau of Investigation, Forensic Services, 690 Kipling Street, Lakewood, CO 80215*

The goal of this presentation is to provide attendees with the facts and circumstances surrounding this case of complex suicide as well as develop a better understanding of the dynamics and patterns involved in complex suicides.

This presentation will impact the forensic science community by providing information regarding complex suicides in general, the facts and circumstances of this case of complex suicide involving a nail gun, and will foster a greater level of understanding of the process involved in the investigation of complex suicides.

Complex suicides are those suicides involving more than one mechanism to inflict lethal injury or cause death. These methods can be used simultaneously or sequentially, when a previous method fails to cause death. The case presented here offers information about an unusual case of complex suicide involving a nail gun.

In this case, a 56-year-old male was found dead in his pickup truck by his wife. The scene investigation disclosed a dryer vent hose was used to route the vehicle's exhaust into the cab of the truck. In addition to the dryer hose, a nail gun was found in the cab of the truck. The nail gun was connected to an air compressor located near the truck. The victim had sustained numerous penetrating injuries to the chest and one to the forehead. There was minimal blood discovered during the scene investigation. A review of the victim's medical and mental health history failed to disclose any history of mental illness. Inside the residence, personal papers including copies of life insurance documents, a Bible, and personal belongings arranged neatly on a table support the conclusion that the victim took his own life. The suicide was most likely triggered by discovery of suspected criminal activity on the part of the victim when an audit disclosed that his business accounts contained unexplainable irregularities.

What makes this case unique is the use of a nail gun as a method of inflicting potentially lethal injury in addition to routing engine exhaust into the victim's vehicle and the consumption of large quantities of ethanol. While the use of power hand tools to inflict injury is not rare, a review of the available literature suggests that use of a nail gun used as a method of inflicting injury is very rare, particularly when used in conjunction with carbon monoxide and ethanol poisoning. In this case, the cause of death was clearly established, penetrating trauma by the nails to the chest and head. A review of the case facts demonstrates how a careful scene investigation and a complete forensic autopsy can help clarify the circumstances of any equivocal death, particularly suspected suicides.

Complex Suicide, Suicide, Nail Gun

E20 Method Development and Optimization of Detection of Decomposition Products in Soil Using Headspace/Gas Chromatography/Mass Spectrometry (HS/GC/MS)

Amanda L. Haggerty, BS, Arcadia University, 450 S Easton Road, Glenside, PA 19038; Kimberlee S. Moran, MSc, Forensic Outreach, 231 Cedarbrook Road, Sicklerville, NJ 08081; and Heather L. Harris, MFS, JD, PO Box 43626, Philadelphia, PA 19106*

After attending this presentation, attendees will better understand the compounds present in soil as a result of decomposition and the application of HS/GC/MS to detect and identify five decomposition products: dimethyl disulfide, heptanal, 1-hexanol, nonanal, and 1-Octen-3-ol.

This presentation will impact the forensic science community by providing additional analytical options to laboratories looking to identify human decomposition. The methods utilized in this study can be applied when searching for clandestine graves.

For years, detection of clandestine burial sites has relied on the use of Volatile Organic Compounds (VOCs); however, without specific instrumentation, detection of VOCs can be difficult. This research investigates the possibility of using HS/GC/MS to detect VOCs in a soil matrix. With little to no sample preparation, HS/GC/MS is a faster and easier method when trying to detect decomposition products. Piglets were used as a proxy for human cadavers and were obtained from the University of Pennsylvania Swine Unit in Kennett Square, PA (*n*=60). All pigs died of natural causes so that interactions with euthanasia drugs were avoided. The piglets were buried at four depths (0.5ft, 1.0ft, 1.5ft, and 2.0ft) in a wooded study area at 10ft horizontal intervals. Soil was sampled from around the piglets at three decomposition states (early, mid, and advanced) determined with the use of the Megyesi method and Accumulated Degree Days (ADDs).[1] ADDs were calculated from the time each piglet was buried. The target ADDs for early decomposition was 340.408. The targets for mid and advanced decomposition were 916.220 and 2387.811, respectively. All pigs were buried in an orientation with the snout pointed west and feet orientated south. At the time of burial, each piglet was given a unique identification number used to log weights and organize samples.

The five VOCs of interest in the project were dimethyl disulfide, heptanal, 1-hexanol, nonanal, and 1-Octen-3-ol. Based on previous literature research, these five compounds were found to be, in both pig and human decomposition, in higher frequencies. Since piglets were being used as a human cadaver substitute, having this crossover can relate back to the compounds that a decomposing human body will release.

Once the piglets reached the required ADDs, a 33-inch AMS® unplated soil probe was used to collect samples. A two-inch section of soil, weighing approximately 27g, was taken from the tip of the soil probe after it reached the correct depth. The depth of each sample depended on the depth the piglet was buried. For example, samples were taken horizontally from six inches below the surface for piglets buried at six inches. These soil samples were then placed in cut pieces of foil to avoid evaporation of the desired compounds. Each sample was labeled with the sample orientation (North or South of the carcass), the specimen number, and the distance from the piglet (0.5ft, 1.0ft, 1.5ft, and 2.0ft). Samples were then placed on ice for transport until they could be placed into a freezer for storage.

Samples were analyzed on an Agilent® 6890 HS/GC using a CTC Analytics® Combi Pal autosampler coupled with an Agilent® 5973 *Network* Mass Selective Detector. Approximately 1.5g of soil was placed into a headspace autosampler vial and analyzed. Aliquots were taken randomly from the collected soil samples and vortexed for approximately 30 seconds to homogenize. All compounds were successfully separated with good resolution and peak shape using the HS/GC/MS.

Reference(s):

1. Megyesi M., Nawrocki S.P., Haskell N.H. Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. *J of Forensic Sci* 2005;5 (3): 618- 626.

Decomposition, HS/GC/MS, Soil

E21 A Follow-Up Study: Recovery of "Touch" DNA From Cleaned Pistol and Ammunition Surfaces

Maher Noureddine, PhD, 5687 Wolf Ridge Court, Oak Ridge, NC 27310; and James A. Bailey, PhD, Minnesota State University Mankato, 617 Chestnut Street, Wilmington, NC 28401*

After attending this presentation, attendees will be familiar with collecting DNA samples from firearms and ammunition and with the impact of cleaning a pistol and ammunition for the detection of residual DNA from a single shooter.

This presentation will impact the forensic science community by describing a pistol and ammunition cleaning method, the effect of cleaning on distribution of DNA profiles on different pistol parts and ammunition, and the implications for a method of universal firearm swabbing.

The quantity and quality of DNA recovered from firearms can vary based on many factors such as the type of firearm, frequency of handling and cleaning surfaces of firearms, cleaning method, physiology of the handler, number of contributors, and downstream testing methods. It is exceedingly difficult to account for such complex variables in any experimental design. In order to provide meaningful guidance to criminal investigations, testing complex variables must be coupled with a comprehensive understanding of the mechanisms by which DNA gets deposited on firearms as well as removed from firearms. A previous study found that full DNA profiles of the shooter can be recovered from swabbing a pistol fired and stored without cleaning for a period of two weeks before swabbing. This study evaluates whether a pistol, which has been in ordinary use, can still yield DNA profile information following one round of thorough cleaning. This study also evaluates whether a single interaction between the shooter and the cleaned pistol will result in a detectable difference in the level of DNA found on the pistol.

A 9mm Smith & Wesson® Model 5906 pistol was handled and fired by one right-handed shooter. After cleaning and storage for a period of two weeks, it was swabbed. The same male subject who fired the pistol removed ten 9mm full metal jacketed cartridges from a new unopened box of American Eagle® ammunition, loaded a full magazine, then inserted and ejected the magazine into the pistol. The pistol, magazine, cartridges, and ammunition box were cleaned using sterile tissues pre-wetted with a cleaner (CLOROX® Clean-Up®), followed by cleaning with sterile tissues pre-wetted with 75% ethanol. Various pistol surfaces, empty magazine surfaces, ammunition, and the ammunition box were then swabbed for DNA. The pistol was given to the shooter who loaded the magazine with the ten cleaned cartridges, the shooter inserted the magazine into the pistol (slide was not retracted), and held the pistol for a period of one minute, simulating an imminent firing posture with his finger touching the trigger. Following this interaction, all surfaces were swabbed again for DNA. All samples and appropriate controls were collected using the COPAN® Crime Scene 4N6FLOQSwabs™ that were pre-wetted with sterile water. DNA samples were extracted using the COPAN® Nucleic Acids Optimizers (NAO), a semi-permeable basket, which retains fluid until centrifuged with the PrepFiler® Express™ on the *AutoMate Express*™ DNA Extraction System. DNA quantitation was performed using the Quantifiler® Human DNA Quantification Kit. The AmpFLSTR® Identifiler® Plus PCR Amplification Kit was used for DNA amplification, the fragments were run on the Applied Biosystems® 3130 Genetic Analyzer, and the analysis was performed with GeneMapper® ID-X v1.4.

Partial Short Tandem Repeat (STR) profiles were detected on the cleaned pistol, magazine, ammunition box, and cartridges; however, the identity of the shooter could not be determined from the majority of partial STR profiles due to potential low-level DNA contamination. A noticeable improvement in the STR profile data was observed on certain parts of the grip and magazine after the shooter handled the pistol once following cleaning. Improvements were not found on the trigger, slide release, frame, hammer, and cartridges.

This study demonstrates DNA profile data can be detected on certain parts of firearms and ammunition after undergoing one round of surface cleaning. Practitioners are cautioned that universal surface swabbing of a firearm might not be as effective as swabbing separate areas. While universal surface swabbing can maximize the amount of total DNA collected, the probative value from such samples can diminish due to the possible creation of artificial mixtures that can render any profile data from a firearm useless.

Touch DNA, Cleaning Firearms, Firearm DNA

E22 Bloodstain Evidence of Trophy Taking in a Homicide

Bryan R. Burnett, MS, Meixa Tech, PO Box 844, Cardiff, CA 92007-0844*

The goal of this presentation is to describe unusual bloodstains generated by the assailant manipulation of the dying victim of a shooting.

This presentation will impact the forensic science community by helping attendees be able to recognize unusual bloodstains, albeit likely quite a rare occurrence.

The homicide of Daniel Lyons occurred in Santa Barbara, CA, May 4, 2009, in the early hours of the morning. Lyons was awakened by the sound of shots being fired at his wife on the first floor of their home. Lyons was in a bedroom on the second floor. Quickly following his wife's murder, an assailant was in his bedroom and fired two shots with a .38 caliber revolver. A second shooter with a 12-gauge shotgun then joined the first assailant. As the room was dark, neither shooter could effectively target Lyons and deliver an immediately fatal shot; most shots missed. Five .38 caliber and four shotgun discharges occurred. Following the shooting, Lyons was still standing despite a .38 bullet wound to his head and shotgun wounds to his abdomen and right hand. Remarkably, after being severely wounded, bloodstains on his body and on the carpet show Lyons struggled with one or more of his attackers, being twice stuck on his head, likely by a hatchet.

The physical struggle between Daniel Lyons and his assailants ended when he lost consciousness, due either to the bullet wound to his head or to the loss of blood from the shotgun wounds to his abdomen and right hand. It was at this time that the assailants repositioned his face-up, naked body. But the manipulation of his body did not stop with Lyons' final position at the scene. It appeared a cloth (pillow case?) was placed over Lyons' abdomen to his lower thighs and was tucked between his legs. Lyons' still-bleeding right hand was placed on the cloth and repositioned several times, soaking the cloth with blood at each location. The assailants took the cloth, but evidence was left on the victim's body of this unusual activity.

To validate this scenario, a mannequin was draped with cotton fabric. Simulated blood was poured over the cloth.[1] The simulated bloodied cloth was removed and the stains left on the mannequin were compared to the bloodstains on the body of Lyons. There were a variety of bloodstain patterns on the anterior body that appeared to have been created by the covering cloth, ranging from a heavy coating of blood over the mid right thigh to sparsely distributed blood on the upper right thigh. In the sparsely bloodied areas on the body, blood that had penetrated cloth also left patterns on the skin somewhat reflective of the cloth's folds as well as highly irregular-shaped stains of different sizes. Distinctive multiple blood streams on the right side of the body occurred from the edge of the cloth on the anterior body to the floor. Most of the bloodstains on Lyons' body were simulated on the mannequin by the cloth soaked with the fake blood.

It is apparent from the covering of the body with a cloth and purposely bloodying it as well as taking it from the crime scene that the assailants were in no hurry to flee the crime scene after the homicides. It is likely that at least one of the assailants was a street gang member who took the bloodied cloth with him as a "trophy" upon leaving the crime scene.

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Bloodstains, Homicide, Trophy Taking

E23 Multidisciplinary Approach to the Identification of Military Remains — An Australian Perspective

Donna M. MacGregor, MSc, Queensland University of Technology, School of Biomedical Sciences, Faculty of Health, Gardens Point Campus, Brisbane, Queensland 4001, AUSTRALIA; Marc Oxenham, PhD, School of Archeaology and Anthropology, Australian National Unversity, Canberra, ACT 2000, AUSTRALIA; Henry Y.H. Wu, Forensic and Scientific Services, Forensic Odontology/Forensic Pathology Unit, Coopers Plains, Brisbane, Queensland 4113, AUSTRALIA; and Brian Manns, Unrecovered War Casualties-Army, R1-4-A113a, Dept of Defence, PO Box 7902, Canberra BC, ACT 2610, AUSTRALIA*

After attending this presentation, attendees will be aware of: (1) the role and capabilities of the Australian Army with regard to locating and identifying the remains of Australian servicemen who remain unaccounted for from past conflicts on foreign soils; and, (2) the multidisciplinary team approach to facilitate the identification of remains.

This presentation will impact the forensic science community by increasing awareness of the Australian Army's capacity with regard to accounting for Australia's missing servicemen. This presentation will also highlight the utility of a multidisciplinary approach to achieve this task, without which a recent identification could not have been substantiated.

The Unrecovered War Casualties-Army (UWC-A) is a relatively new unit within the Australian Defense Force, established in July 2006. Its mission is to account for Australian soldiers who remain unaccounted for from all past conflicts. The role of UWC-A is to: (1) investigate all notifications relating to the discovery of human remains believed to be those of Australian soldiers; and, (2) investigate information that may lead to the discovery of the remains of Australian soldiers. This mission statement is similar to other organizations; however, the uniqueness of this work is that it is achieved by three permanent public service staff, located at Army Headquarters in Canberra, with the remaining team members located across the country serving only in a part-time capacity. The part-time team members are investigators and forensic specialists drawn from the greater forensic community. These specialists are derived from the fields of anthropology, odontology, pathology, archaeology, crime scene, and molecular biology.

In July 2011, a notification to UWC-A resulted in the deployment of a multidisciplinary team to a remote site located at Eora Creek along the Kokoda track. The Kokoda campaign, July and November 1942 (WWII) in Papua New Guinea, occurred when the Australian militia halted the advancing Japanese Imperial Army, causing them to retreat. This campaign sustained heavy casualties for both armies. In a gravesite at Eora Creek, a near-complete set of human remains was recovered and returned to the Australian Commission in Port Moresby for further examination by anthropologists and an odontologist. Based on the quantitative and qualitative examination, the remains were consistent with a young adult male; however, determination of ancestry posed some issues. Initial quantitative assessment of the cranium established that the remains were most likely Caucasoid; however, the central maxillary incisors were heavily shoveled. Using FORDISC® 2, the quantitative assessment of the crania established the remains to be Caucasoid male with a Posterior Probability (PP)=0.397 and Typical Probability (TP)=0.470. Other near possibilities were Japanese male (PP=0.204,TP=0.283) and Hispanic male (PP=0.292, TP=04.29), though given the context, the Hispanic male was unlikely. Stature was initially reported for both a Caucasoid male as 173.4cm±3.27cm and a Japanese male as 167.2cm-171.3cm. The average height for an Australian male during 1942-1943 was 170.2cm-173.7cm and 160.3cm-161.8cm for a Japanese male. Subsequent DNA analysis was performed and ancestry was determined to be East Asian.

Artifacts located within the gravesite surrounding the body were Japanese in origin. Most of the clothing was degraded; however, the boots were more likely Australian-uniform issue. The artifacts included a stamp pad and personalized stamp. This stamp and the biological results, including the mitochondrial DNA (mDNA), subsequently assisted the case investigator to establish the identity of the remains.

The presentation will discuss the case in more detail. This presentation highlights the necessity of a multidisciplinary approach to identification of military remains. Without this approach the discrepancy between the various observations reported by each specialist could not have been resolved and the identity established.

WWII Skeletal Remains, Identification, Australia

E24 "We Didn't Start This Fire" — Understanding What Caused the Fire That Killed Twin Boys

Matthew C. Wietbrock, BS, 629 N 6th Street, Lafayette, IN 47909*

After attending this presentation, attendees will understand the details surrounding a devastating house fire, set under suspicious circumstances, which claimed the lives of 3-year-old twin brothers in April 2014. Attendees will also learn the value of a multidisciplinary approach to such investigation.

This presentation will impact the forensic science community by detailing forensic tasks, which occurred as part of the criminal investigation, as well as the crime scene investigative strategies which were employed. A review of this investigation will demonstrate to attendees the importance of a multidisciplinary approach and effective teamwork to ensure a complete and accurate investigation.

At 11:01 a.m. on April 5, 2014, the Tippecanoe County Dispatch Center was advised of a working house fire in progress in rural Tippecanoe County, IN. County sheriff deputies, the fire department, and ambulances were dispatched to the area.

Upon arrival, first responding officers found the adult occupants of the home outside, with visible burns. The male was distraught screaming that "his babies" were still in the home. Deputies attempted to enter the house, and after several failed attempts to locate a point of entry, located a window covered on the exterior by black plastic. Upon removing the covering, the deputies saw, through the smoke, one of the boys lying on a bed, then immediately saw his brother on the floor. They smashed open the window, resolving to rescue the children. While his partner held onto his feet, a deputy dove into the burning house, pulled both boys out, and rushed them to the waiting ambulances. Both children died upon reaching separate area hospitals.

After the blaze was extinguished, the questions asked were: how did this fire start and what were the contributing factors? The father would later claim that he believed that his boys, perhaps playing with a lighter, had set the house on fire.

Also in question was what the actions of the adult occupants of the house were after the fire began. Witnesses claimed that they observed the father bringing items out of the residence, prior to first responders arriving. Perhaps he even made several trips. All the while, his sons were still inside the home. How did the adult occupants find their way out of the blaze, while the children were left behind? Was the fire set in conjunction with some other criminal enterprise? Also needing to be answered was the question of how to identify the twin bothers and tell them apart from each other for post mortem identification.

A complete scene investigation was critical to answering these questions and, as it turned out, one visit to the scene would not be enough for investigators. With determination and a willingness to question their assumptions, investigators were able to place blame upon the person responsible and, perhaps what is more important, deflect the blame from two innocent victims.

This presentation details those efforts, as well as the eventual conviction of the father of the deceased boys. This conviction would never have happened without a team-oriented, multidisciplinary approach on the part of the Fire Marshal's Office, the Tippecanoe County Sheriffs Department, the pathologist, the Tippecanoe County Prosecutors Office, and the Tippecanoe County Coroner's Office.

Fatal Fire, Multidisciplinary, Neglect

E25 Perceptions of the "CSI Effect" by New York State Prosecutors and Forensic Science Requests at Trial

Elizabeth A. Erickson, MS, 201 Lenny Road, Potsdam, NY 13676*

After attending this presentation, attendees will better understand the definition of the "CSI effect," how prosecutors in New York State view the phenomenon, and whether or not trial modification occurs with an increase for forensic science requests and forensic expert witnesses during trial.

This presentation will impact the forensic science community by examining how an upsurge in forensic science requests from the District Attorney's Office can contribute to an increased backlog at forensic centers.[1] This study expanded on previous research addressing the "CSI effect" from the standpoint of jurors and judges and instead focused on modifications made by the prosecutor during the trial to compensate for the phenomenon.[2,3]

In 2005, the New York State Prosecutors Training Institute developed a workshop to address the "CSI effect" and provide recommendations on how cases could be amended to account for jurors expecting more forensic science and forensic expert witnesses.[4] In recent years, other states, including Arizona and Ohio, developed similar programs to determine how to address the perception of the "CSI effect" among prosecutors and forensic scientists.[5] Of major concern is the increase in forensic laboratory requests that may be associated with the "CSI effect." In 2009, there were 4.1 million requests for forensic science processing, creating backlogs within the system.[6] For DNA processing alone, there were more than 900,000 backlogged cases, which was an increase of nearly 18,000 since 2008.[6] It was assumed that a relationship existed between the perception of the "CSI effect" by New York State prosecutors and an increase in requests for forensic evidence processing and forensic expert witnesses.

In this study, 21 District Attorney's Offices, one-third of the offices in New York State, participated in the research study. Two hundred thirty-six prosecutors, including the District Attorney, were sent the 15-question electronic survey with 136 completions. The survey was divided into three sections dealing with the perception of the "CSI effect," forensic evidence, and forensic expert witnesses. Each category was then subdivided into an overall perception of the "CSI effect" and then the perception based on DNA, fingerprints, and trace evidence. DNA was defined as any biological processing requests, including blood, semen, saliva, and vaginal secretions. Fingerprints were defined as any requests for latent print processing and comparison of fingerprints manually or through the use of the Automated Fingerprint Identification System (AFIS). Trace was defined as any request for evidence associated with hairs, fibers, paint, glass, and footwear analysis.

Statistical analysis revealed a relationship existed between the perception of the "CSI effect" and an increase in requests for forensic evidence processing (p-value=0.0005) and forensic expert witnesses (p-value=0.427). When analyzed further, a relationship did not exist when the perception of the "CSI effect" was associated with specific categories of DNA (p-value=0.1233, p-value=0.6887), fingerprints (p-value=0.5975, p-value=0.6294), and trace evidence (p-value=0.1452) for both forensic evidence and expert witness testimony. A relationship did exist between the perception of the "CSI effect" and increased requests for trace evidence processing (p-value=0.035).

In conclusion, this study provided evidence that prosecutors in New York State perceived that the "CSI effect" existed and subsequently altered trial preparation accordingly with increased requests for forensic evidence processing and increased requests for forensic expert witnesses; however, since an increase does not exist when analyzing specific forensic science disciplines, future training for prosecutors in New York State should concentrate on forensic science disciplines, as opposed to broad overviews of the "CSI effect."

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"CSI Effect," Prosecutors, Forensic Science

E26 How Abductive Reasoning Impacts Criminal Investigations

Lyndsie N. Ferrara, MS, Duquesne University, 600 Forbes Avenue, Pittsburgh, PA 15219; and James B. Schreiber, PhD, Duquesne University, 600 Forbes Avenue, Pittsburgh, PA 15282*

After attending this presentation, attendees will understand the relationship between the six modes of abductive reasoning and criminal investigations.

This presentation will impact the forensic science community by highlighting the importance of abductive reasoning during criminal investigations and provide educational tools to enhance these reasoning skills.

In legal and investigative research domains, abductive reasoning has been used and discussed and computerized programs have even been developed.[1,2] This research explores the six modes of abduction in relation to reasoning patterns of criminal investigators in an effort to better develop reasoning-in-practice skills for forensic science education students. Peirce argued that "All the ideas of science come to it by way of abduction. Abduction consists of studying facts and devising a theory to explain them."[3] The six modes of abduction are based on Peirce's first six classes of signs. The six abductive reasoning modes are: Omen/Hunch; Symptom; Metaphor/Analogy; Clue; Diagnosis/Scenario; and Explanation.

Analysis of two exemplar homicide investigation cases are used in this study. The first case examines the murder of Dr. Jeffrey Farkas, a 26-year-old pediatric intern at Children's Hospital in Pittsburgh, PA, who was found brutally murdered in his home. The convicted murderer, William Yarbough, was sentenced to life in prison. The second case example is based on historical documentation and is known as the "Dutch Case of the Ball Point Pen Murder." In 1991, a woman's son found her lying dead on the floor of the living room in her house. Her right eyelid was swollen and slightly wounded. An autopsy performed the next day found a complete ballpoint pen that had penetrated her eye, causing mortal brain damage.

A comparison of these two cases highlights the importance of abductive reasoning during criminal investigations and highlights ways education can help enhance these skills. In the Dr. Farkas case, the criminal investigation team focused on the evidence and began to build that evidence together from hunches, to clues, to analogies, to potential scenarios of who and why. In the ballpoint case, the investigators focused on an explanation, the end stage of abduction, of murder almost instantly. The case then fixated on finding evidence to support the explanation and not the reverse. The investigators appeared to start with induction and deduction instead of developing multiple potential scenarios and then testing them, as is common with abduction.

This study demonstrates the reasoning processes that occur in criminal investigations and the importance of using abductive reasoning as a primary investigative tool. This is more than basic pattern searching, which can lead to incorrect inferences.[4] The key is the development of the pattern and then the testing of that pattern with new data (evidence). This type of Peircean experimentation is the skill set that needs to be developed and understood to be used to its fullest capacity during investigations. In addition to this work, related work is ongoing in forensic education with reasoning, decision points, and the ethical consequences associated with them.

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Criminal Investigation, Reasoning, Education

E27 A Barrel-Bullet Comparison of Rifling Lines: A Transformation for a Quantifiable Examination Approach

John Z. Wang, PhD, 18737 W Place, Artesia, CA 90701*

After attending this presentation, attendees will better understand an innovative method of comparing rifling lines between a weapon barrel and a fired bullet based on three technical aspects: (1) if the direction of rifling lines inside the barrel has the same direction as that of the fired bullet; (2) if the land shape inside the barrel is identical with that of the fired bullet; and, (3) if the width of the land/groove is identical with that on the fired bullet.

This presentation will impact the forensic science community by providing a project result and answer the challenges from the National Academy of Sciences (NAS) 2009 Report, Strengthening Forensic Science in the United States: A Path Forward, which states that the firearms examination is "less scientific." This study was conducted based on a murder from an actual appellant case in a southern state, in which one of the key issues was to confirm whether a .38 Super pistol or a .38 Special revolver was involved. It is contended that the result is able to provide a novel method for the attendees' future firearm examinations at a crime scene, in the laboratory, and even in courtroom testimony as to whether the bullet found at the scene was fired from the weapon involved.

The firearms examination is challenged by the NAS Report on three major fronts. First, the current examination compares striations only on a fired bullet with these on a test-fired bullet, or the agreement of the striation of the pattern and the minutia. Second, if the fired bullet is retrieved from dirt/sand or walls, many of the striation details on the bullet surface will more likely be lost or damaged, thus making the pattern and the minutia comparison more difficult. Finally, the Report alleges that the examination is "less scientific" due to its nature of being a pattern/minutia comparison, lacking a quantifiable measurement. The Report implies that a conclusion of identification must be made based on a scientific and an objective verification, not on a subjective decision such as patterns and minutia. To qualify as a scientific examination, a conclusion must have an empirical support, an objective methodology, and a quantifiable basis. This presentation provides a preliminary result based on an actual criminal case, using a random sample and employing a digital technique.

It is argued that this study is intended to answer the above three challenges using the 21st-century technology with three unique features: (1) it is a non-destructive method that can be used either before or after the standard examination for either an examination or a verification (a second opinion); (2) the technique can provide a quantitative measurement of the barrel and the bullet, making the examination more reliable and valid; and, (3) the new application can produce a rapid examination at the scene, in the laboratory, and even in the courtroom, adding much-needed support for crime scene technicians and investigators.

The collection occurred in an indoor shooting range based on a subcategory of a random methodology: the target sampling. The actual examination was conducted at a state university campus due to its being a pilot experiment. The preliminary results indicate three findings: (1) contrary to the traditional examination between a bullet and a test fire, this novel method compares a barrel with a bullet, providing a new dimension; (2) instead of the subjective evaluation of images from a comparison microscope, the barrel-bullet comparison is based on a digital examination with up to nine geometric measurements; and, (3) the new method is able to measure the direction of rifling lines, the shape of the land, and the width of the land/groove. If certain conditions exist, this innovative method can even measure the pith of rifling lines inside the barrel and on the bullet, making this a more sufficient and practical examination than traditional examinations. It is argued that if the method were to be additionally tested and finally adopted, our duties and performance will be positively transformed and measured.

Firearms Examination, Rifling Lines, Digital Examination

E28 Forensic Podiatry — How Gait, Footwear, and Footprints Convict Criminals

Michael S. Nirenberg, DPM, Friendly Foot Care, PC, 50 W, Crown Point, IN 46307*

After attending this presentation, attendees will understand the value of forensic podiatry with regard to the interpretation and analysis of pedal- and gait-related evidence encountered at crime scenes.

This presentation will impact the forensic science community by providing insight and knowledge of the exciting, new, and emerging field of forensic podiatry, which can provide valuable, often overlooked, interpretation of pedal- and gait-related evidence, including footwear, human remains, and footprints, in criminal and civil matters.

Forensic podiatry is a subspecialty of podiatric medicine that offers investigators an exciting new tool in the analysis of pedal and gait evidence in criminal and civil matters. Forensic podiatry has been described as "… the application of sound and researched podiatry knowledge and experience in forensic investigations, to show the association of an individual with a scene of crime, or to answer any other legal question concerned with the foot or footwear that requires knowledge of the functioning foot."[1]

Forensic podiatry allows interpretation and analysis of footprints to provide an estimation of the person's sex, height, and other anatomical and biomechanical features, allowing forensic podiatrists to establish a link (or show a lack of association) between a footprint and the perpetrator of a crime. Footprint placement, including such aspects as step length and stride, may furnish information about the person who made the footprints. Footprints at crime scenes are often bare or sock-clad, and may be partial or complete.

Discarded footwear found at crime scenes can provide similar information and, by forensic podiatry analysis, can be linked to and provide information about the wearer of the footwear.

In cases of dismembered human foot remains, forensic podiatry can assist in establishing the victim's identity. Often shoes or boots provide protection for feet, allowing them to be the only surviving aspect of a person in a mass fatality, such as an explosion. A dismembered foot may be linked to the footwear, medical records, and/or radiographs of the suspected victim.

Perpetrators captured on surveillance video whose faces are hidden or not visible can be identified by their gait. The use of gait to assist in the identification of criminals is growing as video surveillance is increasing and devices that enable recording of crimes are becoming more widespread.

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Forensic Podiatry, Footprints, Gait

E29 Use of Unmanned Aerial Vehicles (UAVs) for Documenting the Forensic Scene and Body Retrieval in a Case of Mid-Air Collision Between Aircraft

Angelina I. Phillips, MD, MUSC, 165 Ashley Avenue, Ste 309 MUSC908, Charleston, SC 29425; and Lee M. Tormos, MD, Medical and Forensic Autopsy, Pathology & Laborat Medicine, 171 Ashley Avenue, MSC908, Charleston, SC 29425-9080*

After attending this presentation, attendees will understand the use of UAVs in documenting forensic scenes.

This presentation will impact the forensic science community by demonstrating the benefit of UAVs in documenting outdoor or large forensic scenes.

The UAVs, commonly referred to as drones, are generally known for their use in the military; however, there are a number of law enforcement situations in which they have been effectively utilized. There has been reported use of UAVs to assist the investigation and documentation of crime scenes in several states including North Dakota, Illinois, and Colorado. The UAVs are capable of capturing highly detailed images every two seconds; these are then condensed into a single highly detailed image via computer software. The conglomerated image provides excellent mapping of a large area and can be manipulated to produce a 3D replica of the scene that can be viewed from a variety of angles, allowing for detailed scene recreation/documentation. The UAVs are also capable of real-time video images, which are particularly useful as guides for identifying and localizing objects or persons of interest in search and rescue operations.

This study illustrates a case in which UAVs were used to collect multiple images and data points to map the scene after the mid-air collision of an F16 fighter jet with a Cessna® airplane. The data provided by the UAVs not only assisted with documentation of the scene but also in identifying areas to focus the search for the remains of the two individuals in the downed plane. Based on eye-witness information gathered by the Beaufort County coroner, the collision of the F16 with the Cessna® produced an extensive debris field spanning approximately 2,000 square meters over water, a previous rice field, and wooded terrain. The Skyview Aerial Solutions company was contacted and were on scene with three UAVs that they used to help identify the site of major fuselage debris, which was not in easily accessible terrain.

Despite the proven usefulness of UAVs for law enforcement endeavors, UAV licensing and permitted use is still under consideration due to possible interference with aerial rescue vehicles and because their unrestricted use may incidentally provide surveillance information not pertaining to the investigation, which legally cannot be gathered without a search warrant.

Most commercial, civil, and private use of UAVs falls under the jurisdiction of the Federal Aviation Authority (FAA), who in 2012 drafted the "Federal Aviation Authority Modernization Act" which clarified the position of the FAA, was the first to define unmanned aircraft, distinguished between the different types, and required the development of regulations for safely integrating civil UAVs into the national airspace system. With an estimated 7,500 small commercial UAVs anticipated to be in operation by 2018, the FAA expects to have fully developed regulations for their use by 2016. In the interim, at least 20 states have passed legislation directly regarding the use of UAVs and the data which they collect for the purpose of law enforcement.

Shortly after a major incident such as natural disaster, mass casualty event, or in this case, mid-air collision over difficult terrain, the disaster area can be chaotic. A scene can often consist of expansive distances, and as such, evidence may be difficult to identify, recover, become lost, or even destroyed. The future use of UAVs may be an advisable and beneficial tool for large outdoor scene documentation to be utilized by those in law enforcement.

Unmanned Aerial Vehicles, Drones, Scene Documentation

E30 Suicidal Hanging: A Prospective Autopsy-Based Study of 650 Cases

Mantaran Singh Bakshi, MBBS, All India Institute of Medical Sciences, C - 78, Defence Colony, New Delhi 110024, INDIA; Sudhir Kumar Gupta, MD, AIIMS, Dept of Forensic Medicine & Toxicology, New Delhi 110029, INDIA; Deepak Prakash, MD, All India Institute of Medical Sciences, Rm No. 605, Masjid Moth Resident Doctors Hostel, New Delhi 110049, INDIA; Piyush Sharma, MD, All India Institute of Medical Sciences, Rm No-302, Dept of Forensic Medicine, New Delhi, Delhi 110029, INDIA; and Shivani Dhaka, MBBS, AIIMS, New Delhi, Deptof Forensic Medicine, AIIMS, New Delhi, Delhi, INDIA*

After attending this presentation, attendees will better appreciate the importance of gross and significant microscopic findings in a case of suicidal hanging and will appreciate the underlying reasons for suicide.

This presentation will impact the forensic science community by presenting cases of fatal pressure on the neck in suicidal hangings. This presentation will add to the relevant histopathological findings as well as introduce new findings and even disprove outdated and doubtful assumptions.

Hanging is the leading method of suicide in India.[1] Of all asphyxial deaths, difficulties most commonly arise in distinguishing cases of suicidal hanging from other forms of ligature asphyxia without any obvious classical external findings.

In this study, 650 cases of suicidal hanging were studied. This study was conducted at a tertiary care center in the capital territory of India, New Delhi. The most common age group involved in this study was 15 years to 30 years of age. It was observed that the majority of the cases were male (63%) and the most common ligature material used was a chunni/saree (Indian female attire, as compared to the rope which was seen in other studies).[2-5] Special attention was paid to the ligature mark produced by the clothing materials, as they produce a faint, broad mark with an intervening normal area. The ligature mark was confirmed with an histopathological analysis for signs of inflammation, such as leucocyte infiltration. On microscopic examination of the neck muscles after layer-wise dissection, hematoma and inflammatory findings were seen in the platysma (17%), sternocleidomastoid (10%), mylohyoid (7%), geniohyoid (2%), stylohyoid (12%), and digastrics muscles (1%). The fracture of the thyroid cartilage was not seen in this study, but, surprisingly, a hyoid bone fracture was found in 3% of the cases, which is comparable with the study done by Feigin et al.[6] Amussets sign, a transverse intimal tear of the carotid artery, was seen in 12.5% of the cases, which is comparable with the findings of Hejna.[7,8] A histopathological examination of the sub-mandibular salivary gland perifollicular congestion, a perifollicular hemorrhage, follicular hemorrhage of the sub-mandibular gland was observed in 30% of cases and capsular and cortical hemorrhage in lymph nodes was observed in 32% of the cases.

The psychological autopsy of cases revealed 320 cases had a history of clinical depression, 126 had family strife and the rest suffered from failure due to poor academic performance.

In conclusion, this study sheds light especially on the gray areas in which difficulty arises in differentiating cases of suicidal hanging from other forms of asphyxial deaths, as well as testing the viability of pre-existing dogmas prevalent in the identification of cases of suicidal hanging. This study also touches upon the socio-cultural impact of the manner involved in cases of hanging, which are vastly different from those existing in the western world, and is thus an attempt to amalgamate this knowledge and introduce a new understanding of hanging at a global level. As suicide rates tend to skyrocket and with hanging being the most prevalent method, it is paramount that practitioners set criteria for identifying such cases to reduce the doubtful opinions which can sometime arise after autopsy in these cases. A psychological inquiry into the inciting cause of such cases will help in discovering trigger factors for such incidents and help in formulating future prevention programs.

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Suicidal Hanging, Ligature, Psychological Autopsy

E31 Suicide or Homicide: A Case of Multiple Stab Wounds and Poisioning

Kelly Kraus, BS, 2326 Eagle Creek Drive, Charleston, SC 29414*

After attending this presentation, attendees will understand the importance of a complete and thorough medicolegal death investigation in cases in which the death was due to multiple stab wound injuries and that the manner of death may be a suicide or a homicide. This case study presentation will demonstrate how and why these complex cases must be thoroughly investigated by medicolegal death investigators and treated as a homicide until proven otherwise.

This presentation will impact the forensic science community by providing information regarding suicides involving multiple stab wound injuries and the medicolegal death investigation process used to investigate one such case.

Suicides resulting from multiple stab wounds/multiple injuries are fairly uncommon and much can be learned from those cases when they do occur. This presentation involves a case study of a 36-year-old Caucasian male who died from approximately 15 self-inflicted stab wounds. This case study highlights the importance of a multidisciplinary team approach while investigating these complex medicolegal death investigations that may initially be considered either a suicide or a homicide.

This presentation includes: case information regarding the extensive death scene investigation which includes multiple weapons, multiple locations and the presence of rat poison and other toxic substances; a review of pertinent medical records; a review of social service records; autopsy findings; toxicology results; and the relevant interviews conducted by the medicolegal death investigator and law enforcement personnel who were utilized prior to ruling the death a suicide.

This study discusses the physical findings noted in this case as well as similarities and differences which may be found in suicide deaths resulting from multiple stab wounds and a homicide resulting from multiple stab wounds. The importance of a medicolegal death scene investigation and a complete medical record review will also be discussed. This case study is a useful tool in explaining and discussing the importance of an immediate and timely death scene investigation and the information which may be obtained. This case also highlights the importance of obtaining and reviewing all social records as well as the importance of interviews which may be conducted by medicolegal death investigators and/or law enforcement personnel with family, friends, co-workers, medical providers, and others. A team approach to investigating these complex and suspicious death investigations is required in order to accurately determine the manner of death.

Suicide, Stabbing, Rat Poison

E32 A Case Review of a Suicide by Homemade Miniature Cannon

Meryle A. Dotson, MA, District 5 ME Office, 809 Pine Street, Leesburg, FL 34748; Kyle Shaw, MBBS, District 5 ME Office, 809 Pine Street, Leesburg, FL 34748; and Brett E. Harding, MBA, District 5 MEO, 809 Pine Street, Leesburg, FL 34748*

After attending this presentation, attendees will have reviewed a case of suicide by handcrafted cannon in a rural, residential setting.

This presentation will impact the forensic science community by presenting a unique scenario in which an individual may utilize a non-traditional firearm to commit suicide, even in a community where legal access to firearms is prevalent.

This presentation may assist law enforcement and medicolegal investigators by contributing to the understanding of the manner in which a thorough scene investigation can reveal unconventional methods of injury. In this case, a multidisciplinary approach to death investigation was imperative as a detailed account of the decedent's social and occupational history undoubtedly provided additional insight into the unique method of injury.

Firearm-assisted suicide is the most prevalent method of suicide in the United States.[1,2] While they do occur, instances of suicide by handmade firearms are relatively rare in the United States as the ability to obtain legal access to firearms is widespread throughout most of the country. Suicides by handmade firearms are more frequently reported in countries or communities where access to firearms is limited or restricted, or where economical or financial restraints may prohibit the purchase of a firearm.[1,3] Typically, manually constructed firearms utilized in cases of suicide are reported to mimic the mechanics of low-caliber weapons or shotguns. This presentation will present circumstances of the scene investigation and autopsy findings for an unusual case in which a successful suicide was carried out with a handmade miniature cannon.

The deceased in this case was a 56-year-old male who resided with his wife in a rural, manufactured residence in Florida. The decedent's wife had recently moved out of the residence, and the decedent was known to have a history of depression and suicidal thoughts. While having a conversation with his wife, the decedent suggested that he may harm himself, and his wife notified law enforcement. Law enforcement responded to the residence and found the decedent's body in a bedroom. He was lying in a supine position on the bed with obvious trauma to his face. A cigarette lighter and a miniature handcrafted cannon were found in proximity to the body. A letter to his wife was left in the living room. After subsequent investigation of the circumstances, foul play was not suspected.

The decedent was a skilled welder and subsequent investigation revealed that he had constructed multiple miniature cannons with detachable wheels during the 1980s. Published reports of suicide by handcrafted firearm discuss the common manner in which an individual may construct a firearm for the purpose of committing suicide; however, this case presents a unique perspective, as the handcrafted firearm in this case was not constructed with the intention to cause harm. The decedent constructed the miniature cannons to use at celebratory events and gave one cannon to a family member as a gift. He was known to load the cannon with various materials in order to provide a "bang effect" at weddings or on holidays. While the decedent's reason to choose a handcrafted cannon as a method of suicide over more traditional methods, such as hanging or prescription medication overdose, may not be known, a review of the literature suggests that it is not unreasonable to consider that a well-defined skill set and a degree of professional pride may have played a role.1

This case study illustrates that a thorough investigation of scene circumstances is necessary in order to efficiently identify unconventional methods of injury and that subsequent investigation is crucial to eliminate the possibility of suspicious circumstances when a traditional firearm is not located at the scene. While access to firearms may be prevalent in rural, Florida communities, it is imperative for scene investigators to recognize atypical forms of firearms in cases of suicide in order to exclude homicide as a manner of death. Additionally, it is important for pathologists to gain insight into the unique injuries that may result from handcrafted firearms.

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Suicide, Handcrafted Weapon, Cannon

E33 Cases of Forensic Human Identification Using Hair

Songhie An, 10 Ipchun-ro, Wonju, Gangwon-do 220-170, SOUTH KOREA; Myung-duck Kim, PhD, National Forensic Service, 10, Ipchun-ro, Wonju-si, SOUTH KOREA; Kiwook Kim, MS, National Forensic Service, 10, Ipchun-ro, Wonju-si, SOUTH KOREA; Jin Hee Lee, 10 Ipchoon-ro, Won Ju, Gangwon-do 220170, SOUTH KOREA; Byung-Ryul Song, PhD, National Forensic Service, 10, Ipchun-ro, Wonju-si, SOUTH KOREA; Geummun Nam, PhD, 10, Ipchun-ro, Wonju-si, Gangwon-do, Gangwon-do, SOUTH KOREA; and Jisook Min, 10, Ipchun-ro, Wonju-si, Gangwon-do 26460, SOUTH KOREA*

After attending this presentation, attendees will better understand a pretreatment method for analyzing hair and stable isotope ratio data of hair sufficient for identification as an alternative method for DNA analysis.

This presentation will impact the forensic science community by relating the importance of carbon, sulfur, and nitrogen isotope ratios for discrimination of the victim's hair.

If stable isotope data are going to become an important forensic parameter in the identification of people, it is important to map intra- and inter-individual variations across the globe. Therefore, international co-research throughout Asia, Europe, America, and Africa is necessary. These case reports provide practical examples of applying stable isotope signatures for identification.

Case 1: Hair samples including Evidence 1 (victim's hair), Evidence 2 (hair from the victim's boyfriend), Evidence 3 (hair from the crime scene), and Evidence 4 (suspect's hair) from the crime scene and the suspect were compared. The 100ug-weight samples were granulated in a tin capsule and a carbon isotope ratio was obtained using Elemental Analyzer-Isotope Ratio Mass Spectrometer (EA-IRMS) and a two-point calibration was applied with polyethylene (IAEA-CH-7, International Atomic Energy Agency, $\delta^{13}C$ certified value: -32.15±0.05‰ VPDB) and sucrose (IAEA-CH-6, International Atomic Energy Agency, $\delta^{13}C$ certified value: -10.40±0.03‰ VPDB). Only carbon isotope ratio data was obtained because of the limited amount of the samples. As a result, the carbon isotope ratio data of Evidence 3 and 4 were similar to one another. Therefore, the supposition that this person is not a suspect cannot be excluded.

Case 2: Hair sample (Evidence 1) from a hit and run victim and hair from the suspect's windshield (Evidence 2) were compared. There were no roots attached to the hairs from the windshield. The samples were cleaned and 3cm-long subsamples were analyzed for isotopic composition of light elements (i.e., ^{13}C, ^{15}N, and ^{34}S). The thickness of the hairs from the victim and the windshield were different with the unaided eye. Additionally, the stable isotope ratio data were obtained using EA-IRMS. Evidence 1 and Evidence 2 differed in ^{15}N versus ^{13}C and ^{34}S versus ^{13}C. In conclusion, stable isotope ratio data of hair were sufficient for identification as an alternative method for DNA analysis. Further studies are required to routinely retrieve forensic information that is imprinted in hair, such as dietary habits or recent movements of individuals, to support criminal investigations.

Hair, Stable Isotope Ratio, Identification

E34 Smartphone Uses in Medicolegal Death Investigation

Kathryn H. Haden-Pinneri, MD, Harris Co Inst Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; and Bethany L. Bless, MS, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will better understand the methods in which a smartphone may be utilized in the death investigation process.

This presentation will impact the forensic science community by offering another important tool to use during death investigations.

Telecommunication technology has greatly advanced in recent years, providing users with a wealth of information at their fingertips. According to recent figures, approximately 64% of American adults now own some type of smartphone and the number of users in the United States will surpass two billion in 2016.[1,2] As a result, encountering a smartphone or electronic device on a decedent or at a death scene is more common than not. Information obtained from these devices can provide important information for medicolegal death investigators and law enforcement personnel. Applicable laws must be followed, though, when accessing an individual's cell phone.

Password-protected devices may prohibit forensic investigators from accessing this important information. Some cell phones, as well as computers, have built-in fingerprint scanners to provide a more secure way to lock and unlock the device. Fingerprint readers on the newer devices work by capacitance and radio frequency. The addition of the radio frequency allows for discrimination between an actual finger and a single-dimension copied fingerprint. This allows the possibility for a decedent's non-decomposed finger to be utilized to unlock a device. As is true with living individuals, cold temperatures may affect the appearance of the print and the ability of the device to read it; therefore, warming of the decedent's finger is recommended. A recent court ruling determined that a person can be compelled to provide their fingerprint to unlock a smartphone because it is considered physical evidence; however, they cannot be compelled to provide a passcode because that is considered knowledge and not a physical object.

Accessing recently sent text messages could provide information as to the emotional mindset of the individual. Suicide notes are being sent as text messages and as social media posts by the younger generations more frequently than handwritten notes. Visualizing the time/date stamp on the devices for calls made and text messages sent can provide information regarding when the individual was last known to be alive. Text messages may also mention or contain photographs depicting drug use, suicidal ideations, or violent tendencies. It is important to take photographs of important text messages or photographs for documentation purposes.

Smartphones can be used by medicolegal death investigators in other ways as well. Scrolling through the contacts on a phone typically provides information on the potential legal next of kin. Most phones now have a contact listed as "ICE" which stands for "in case of emergency." This information should also be documented photographically.

Cell phone use has also been implicated in deaths due to distracted driving or walking. Finding an open cell phone with a partially written text message in the lap of a motor vehicle operator or in the hand of a pedestrian struck by a vehicle could indicate the person was utilizing the device when the accident occurred. Finding headphones in the ears of a pedestrian struck by a vehicle may be an important finding to explain why they may not have noticed a vehicle approaching.

Law enforcement personnel may also be able to obtain important documentation from devices for the death investigator or pathologist assigned to the case. Photographs taken days before or the day of a death may show the absence of injuries and help establish a timeline for the injuries. This is especially helpful with infants and young children. Tracking a device's Global Positioning System (GPS) coordinates may assist with following the individual's movements prior to death. The key is to think about and know how to obtain this information. This presentation will show a variety of case examples illustrating the various ways smartphone technology can assist with a death investigation.

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Smartphone, Death Investigation, Text Messages

E35 A Multidisciplinary Approach to Exhuming a Body Buried Under a Newly Built House

Dae-Kyoon Park, MD, PhD, Soonchunhyang University, Dept of Anatomy, College of Medicine, 31 Sooncheonhyang 6-gil, Dongnam-gu, Cheonan-si, Seoul 31151, SOUTH KOREA; Nak-Eun Chung, PhD, Division of Forensic Medicine, Seoul 158-707, SOUTH KOREA; Yi-Suk Kim, MD, PhD, Ewha Womans University, Dept of Anatomy, School of Medicine, 911-1, Mok5-dong, Yangcheon-gu, Seoul 158710, SOUTH KOREA; U-Young Lee, MD, The Catholic Univ of Korea, Dept of Anatomy, Coll of Med, 505, Banpo-dong, Seocho-gu, Seoul 137701, SOUTH KOREA; Deog-Im Kim, PhD, Keimyung University, College of Nursing, 1095 Dalgubeol-daero, Dalseo-gu, Daegu 704701, SOUTH KOREA; Yong-Woo Ahn, DDS, PhD, Institute of Forensic Medicine, School of Med, Pusan Natl Univ, 1-10, Ami-dong, Seo-gu, Busan 602739, SOUTH KOREA; Taeyeong Kim, NICE Program, 31 Sooncheonghyang 6-gil, Dongnam-gu, College of Medicine, Soonchunhyang Univ, Cheonan-si, Chungcheongnam-do 31151, SOUTH KOREA; and Eungmyeong Kang, NICE Program, 31 Sooncheonghyang 6-gil, Dongnam-gu, College of Medicine, Soonchunhyang Univ, Cheonan-si, Chungcheongnam-do 31151, SOUTH KOREA*

After attending this presentation, attendees will understand the results of multidisciplinary approaches to exhuming a body and recovering physical evidence, which are essential for prosecuting perpetrators of homicide cases.

This presentation will impact the forensic science community by demonstrating how to establish a multidisciplinary approach and international collaboration to exhume a body that was buried under a newly built house in a foreign country.

From 2005 to 2010, several Korean travelers were reported to have been kidnapped, murdered, and buried in the Philippines by a Korean gang. The Korean National Police Agency investigated these cases, but unfortunately did not recover the bodies or other physical evidences. One of the gang members turned himself in and confessed to two murders. The main challenge was that a new house had been built on the burial sites. The Korean government persuaded the homeowner to allow the excavation of the bodies, and the homeowner agreed to the destruction of his living room floor.

The Korean National Police Agency asked the National Forensic Service to recruit experts to exhume the body. The Korean Crime Scene Investigation (KCSI) team consists of seven specialists: a forensic pathologist, a forensic radiologist, a forensic anthropologist, an engineer for Ground Penetrating Radar (GPR), and three crime scene investigators. After scanning with the GPR, two possible burial sites were located, and these sites were excavated until body parts were exposed. When the bodies were found, the Scene Of Crime Officer (SOCO) was summoned to the scene in the Philippines, along with the KCSI, including the forensic anthropologist to assist in the exhumation.

Two skeletonized bodies were recovered, one at each possible site. One was wrapped with bed linen, and the other had both hands tied behind the back. The DNA samples were analyzed by the Korean and Filipino forensic biology divisions. Both victims were positively identified based on the results from DNA, dental, and medical records. The bodies of the victims were repatriated to the National Forensic Service in Korea, and forensic anthropological examinations were performed once more. The bodies were then released to the bereaved family.

When a person disappears, many police agencies initiate a missing person case. In order to establish a murder case, the physical body of missing person must be obtained. Owing to a multidisciplinary approach and international collaboration, the Korean National Police Agency was able to locate the bodies of the two victims and prosecute the gang leader for homicide based on the physical evidence.

Exhuming the Body, Multidisciplinary Approach, Prosecuting Perpetrators

E36 Recognition of Skin Damage Caused by the Presence of Different Insects on Dead Bodies

Carolina Nuñez Vázquez, PhD, Universidad Nacional Autónoma de México, Av. Universidad 3000. Ciudad Universitaria, Mexico City 04510, MEXICO; and Lorena Valencia Caballero, PhD, Licenciatura en Ciencia Forense, Circuito de la Investigación Científica s/n, Av. Universidad 3000, Facultad de Medicina, Ciudad Universitaria, Distrito Federal 04510, MEXICO*

After attending this presentation, attendees will recognize the characteristics of the damage done to the skin of corpses by the bites of ants and cockroaches.

This presentation will impact the forensic science community by showing how to generate information that helps researchers differentiate bites of ants and cockroaches from burns caused by chemical agents and cigarettes.

There are many forensic cases documented in which the presence and activity of insects that feed on the skin were found, either on living people, as in cases of neglect, or on deceased individuals. In the latter case, researchers often are in conflict because they have trouble determining if the damage present on the cadaver skin was caused by insects or are marks of abrasion, damage by chemicals, or even if they were caused by fire exposure, as in the case of burns made by cigarettes. In most forensic cases in which damage by the insect activity that fed on the dead bodies' skin was reported, the reports are not based on studies, but mostly assume or infer insect damage as the insects were found at the site.

With the goal of creating a better tool for identifying insect bites in such cases, this research was conducted in order to create reference patterns of features left by the bites and activity of ants and cockroaches when they feed on the skin of corpses, and then to distinguish these marks from marks made by other agents.

In the laboratory, the characteristics of the damage caused by the activity and biting of ants and cockroaches on the skin of cadavers was evaluated. The experiment was carried out under controlled conditions in a bioclimatic chamber. A fluctuating average temperature of 20°C was used, with a low temperature of 15°C and a maximum of 25°C, with a light exposure of 12hr-day/12hr-night cycle. Species of ant and cockroach colonies were previously established. Twenty pieces of skin were used, each 5cm x 5cm. Ten pieces were exposed to each insect colony. For each type of acid, 5ml each of sulfuric and hydrochloric acid were added to ten pieces of skin. Six pieces of skin were also placed as a control. In all cases, observations were made and photographs taken at different intervals of time (1hr, 4hr, 1d, 4d, 1wk, 2wk, and 1mo). Subsequently, all results were compared through microscopic, macroscopic, and raking light analysis to define the characteristics of the damage caused to the skin due to bites of each species of insect, against the characteristics presented on injury marks caused by exposure to sulfuric and hydrochloric acid, as well as control pieces. This study shows some features that help to differentiate patterns of some insect bites on the skin of the dead bodies from patterns left by sulfuric and hydrochloric acid.

Dead Bodies, Skin Damage, Insects Bites

E37 Elemental Composition of Tattoo Inks as an Identification Tool

Trevor E. Curtis, BS, Michigan State University, 578 S Shaw Lane, Rm 319, East Lansing, MI 48824; John P. Buchweitz, PhD, Michigan State University, 4125 Beaumont Road, Lansing, MI 48910; and Ruth Waddell Smith, PhD, Michigan State University, School of Criminal Justice, 560 Baker Hall, East Lansing, MI 48824*

After attending this presentation, attendees will understand the elemental composition of tattoo inks, the ability to use these compositions to differentiate tattoo inks from one another, and the development of a tattoo ink database containing their compositions and its potential uses.

This presentation will impact the forensic science community by providing further information on the composition of common tattoo inks. As these inks are not regulated in the United States, such information is not widely available although it has the potential to be used in the identification of decomposed or otherwise severely damaged human remains.

The art of tattooing has been practiced for thousands of years and has been used for human identification purposes for centuries. Tattoos have been used to mark slaves, brand criminals, and to show hierarchy in tribal populations. Currently, tattoos are viewed as an art form. Because of this, their popularity has grown drastically in recent years to the point where an estimated one-tenth of the world's population and one-quarter of the United States population brandishes this identifying mark.

Tattoo ink is injected into the dermis, to a depth below that where most damage from cuts and even second-degree burns occur. This gives tattoos a degree of permanence as they are relatively unaffected by superficial changes to the skin. Accordingly, tattoos have the potential to be used to narrow down the possible identifications of severely damaged human remains. For example, the National Missing and Unidentified Persons System currently has 11,429 open missing person cases. The possible identifications drops to 100 people when the system is searched for persons with red tattoos, narrowing the search; however, when severely damaged human remains are found, any tattoos are unlikely to be visible. The elemental composition of tattoo ink has the potential to be used to determine if a tattoo is present and the color of the ink. This information could then be used to further support identification efforts.

The overall objective of this research is to determine the utility of tattoo ink for the use of body identification in various stages of decomposition; however, as the composition of tattoo inks is unregulated in the United States, the identity of the elements present is relatively unknown. Hence, the first step in this research is to determine the elemental composition of common tattoo inks.

To accomplish this, a set of common tattoo inks were purchased and microwave-digested in hydrogen peroxide and nitric acid. The acid digests were further diluted and analyzed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Based on previous reports, nine elements including copper, titanium, and barium were selected for analysis in this initial study. The instrument was calibrated for these elements and analytical figures of merit, including linear range and limit of quantitation, were determined. The inks were then analyzed, quantifying the nine elements present in each ink.

Initial analysis of tattoo inks indicates discernable elemental composition differences between inks of different colors. For example, blue and green inks contain copper at concentrations up to 12.4mg per gram of ink while other colors tested tend to contain less than 0.5mg copper per gram of ink. Likewise, some yellow inks can be distinguished based on high barium concentrations, up to 5.5mg/g compared to 0.005mg/g or less in other inks. This presentation will further discuss the elemental concentrations of tattoo inks and introduce the development of a searchable database containing element compositions of the tattoo inks analyzed thus far.

Tattoo Ink, Element Composition, ICP-MS

E38 Identification of Bullets Fired From Consecutively Manufactured Double-Broached Ruger® SR9c® Barrels Utilizing Comparison Microscopy and Confocal Microscopy

Jennifer L. Stephenson, MSFS, Federal Bureau of Investigation, 2501 Investigation Parkway, Quantico, VA 22135; and Erich D. Smith, MS, Federal Bureau of Investigation, 2501 Investigation Parkway, Rm 4340, Quantico, VA 22135*

After attending this presentation, attendees will be aware of tool marks produced by double-broached Ruger® barrels, the process of conducting a blind validation study, pattern matching error rates, methods for distinguishing between subclass and individual characteristics on test-fired barrels, acquisition techniques for bullets using 3D confocal microscopy, and correlation procedures used to evaluate 3D topographies from test-fired bullets.

This presentation will impact the forensic science community by supporting traditional means of pattern-matching methods for identification and by beginning to establish/develop an objective non-traditional means to evaluate the rendering of an identification. Additionally, this presentation will serve as a confirmation to the firearms/tool marks theory of identification that the extent of sufficient agreement of individual characteristics occurring in tool marks produced by the same tool exceeds that agreement which occurs in tool marks produced by different tools. This presentation will also inform the forensic community about applications of an emerging technology within comparative-based disciplines.

This study was conducted to evaluate tool marks imparted on pistol barrels as a result of a double-broach rifling process. Qualified examiners in the Federal Bureau of Investigation's (FBI's) Firearms/Toolmarks Unit (FTU) participated in this blind study to compare test-fired bullets from double-broached barrels in order to determine if the presence of subclass characteristics is a cause for concern when rendering identifications.

Test fires were examined from 15 double-broached Ruger® pistol barrels. Twelve barrels were manufactured within a single production run of a broach (run 9mm PBS 650), ten being consecutively manufactured (designated CM 0-CM 9) and two selected from further down the production run (designated CM 22 and CM 33). Three barrels/pistols were selected from the FBI FTU Reference Firearms Collection (RFC) and are designated D1893, D1925, and D1994. Pistols D1893 and D1925 are model SR9®. The production run barrels and pistol D1994 are model SR9c®, the compact version of the SR9®. The pistol frame/slide from D1994 was used to fire the production run barrels. One cartridge was fired through each production run barrel by Ruger® prior to this study.

The ammunition selected for this study was Remington® UMC® 9mm Luger®, 115 grain, copper full metal jacket. Ten cartridges were fired from each of the 14 barrels and six cartridges from D1994 to provide a total of 146 cartridges. Cartridges were fired into a ballistic water tank to preserve the condition of the bullets. Cartridges were laser engraved with a unique randomly selected numerical identifier, between 100 and 500, prior to firing.

The first six test fires collected from each barrel (first two cartridges for D1994) were used to provide samples for FTU examiner trainees and other research projects. The seventh through tenth test fires collected from each barrel (third through sixth for D1994) were used to create five individually unique test sets for this study. Each test set contained 12 fired bullets, including at least one matching pair from four or more production run barrels and one or more matching pairs from RFC pistols. Each test set also contained an instruction sheet and an answer worksheet, which insured all 66 bullet comparisons were completed for each test set. This was a blind validation study because test sets were placed into a room where the test administer could not see participants pick-up/return the test sets, participating FTU examiners were not provided with any information in regard to the origin of bullets in the test sets, there were no "knowns," and the nature of the answer worksheet ensured the test administer could not tell which examiner completed the worksheet.

Upon return of the test sets from examiners, a 3D topography of each individual land from every test bullet was acquired using a Sensofar® S neox confocal microscope, which provided a total of 360 (5 tests, 12 bullets, 6 lands each) acquired 3D topographies for comparison analysis. The 3D topographies were analyzed with the application of a Cross-Correlation Function (CCF_{MAX}) that provided an objective numerical value that represents the similarity between two topographies. The numerical values were used to determine if there was significant and sufficient variation of individual characteristics between two test fires to correctly render a conclusion or if there were subclass characteristics present which would prevent a conclusion from being correctly rendered. Results from the examiners' conclusions using traditional comparison microscopy were compared to results obtained using confocal microscopy combined with CCF_{MAX}.

Subclass Characteristics, Firearm Identification, 3D Topography

E39 Surface-Enhanced Raman Spectroscopy (SERS) for the Forensic Analysis of Vaginal Fluid

Kathryn A. Zegarelli, BS, 55 Deerfield Street, #2, Boston, MA 02215; Jennifer Fore, PhD, Boston University, Photonics Center-Dept of Chemistry, 8 St. Mary's Street, Boston, MA 02118; Ranjith Premasiri, PhD, Boston University, Dept of Chemistry, 590 Commonwealth Avenue, Boston, MA 02215; Brandon Scott, PhD, Boston University, 8 St. Mary's Street, Boston, MA 02215; Amy N. Brodeur, MFS, Boston University School of Medicine, Biomedical Forensic Sciences, 72 E Concord Street, R806, Boston, MA 02118; and Lawrence Ziegler, PhD*, Boston University, Dept of Chemistry, 590 Commonwealth Avenue, Boston, MA 02215*

The goal of this presentation is to provide a basic understanding of an emerging technology in forensic analysis known as SERS and how vaginal fluid stains can be identified and distinguished from other body fluids using SERS. This presentation will discuss the effects of aging on the SERS spectra of dried vaginal fluid samples, the molecular basis for these spectral changes, donor variability, and menstrual cycle dependence of the vaginal fluid SERS spectra.

This presentation will impact the forensic science community by showing how this new optical approach has the potential to produce confirmatory, on-site, rapid, and non-destructive identification of vaginal fluid, which is a level of discrimination that has not yet been established. This presentation will also inform attendees as to how this new approach could reduce the time and cost required for specimen analysis.

Vaginal fluid is most often found at crime scenes where a sexual assault has taken place or on clothing or other items collected from sexual assault victims or perpetrators. Because the victim is generally known in these cases, detection of vaginal fluid is not a matter of individual identification as it might be for semen identification. Instead, linkages can be made between victim and suspect if the sexual assault was carried out digitally or with a foreign object (e.g., bottle, pool cue, cigarette, handle of a hammer or other tool, etc.). If such an object is only analyzed for DNA and the victim is identified, the suspect may claim that the victim's DNA is present because she handled and/or is the owner of the object and not because it was used to sexually assault her; identification of vaginal fluid residue would alleviate such uncertainty. Most of the research conducted thus far regarding methods for the identification of vaginal fluid involves messenger RNA (mRNA) biomarkers and identification of various bacterial strains; however, these approaches require extensive sample preparation and laboratory analysis and have not fully explored the genomic differences among all body fluid RNAs.[1-3] No existing methods of vaginal fluid identification incorporate both high specificity and rapid analysis. Therefore, SERS has the potential to improve current vaginal fluid identification techniques due to its ease-of-use, rapid analysis time, portability, and non-destructive nature.

For this experiment, all vaginal fluid samples were collected from anonymous donors by saturation of a cotton swab via vaginal insertion. A procedure was developed to accurately and reproducibly extract the dried vaginal fluid and 1.0µL of the extracts was analyzed on gold nanoparticle chips.[4] This metal substrate is the signal-enhancing factor of SERS that quenches any background fluorescence that would interfere with normal Raman spectroscopy.[5] The small sample volume is a result of the high sensitivity of SERS, especially with dilute solutions, which is ideal in cases with little evidence available for collection and subsequent analysis.

Vaginal fluid signal variation of a single sample over a six-month period was evaluated under both ambient and frozen storage conditions using an optimized extraction method: a small swab cutting (~2mm x 2mm) was placed in 10µL of water, the volume was pipetted up and down five times to agitate the sample, and the sample was allowed to extract for ten minutes at room temperature. Vaginal fluid samples were also taken from multiple individuals over the course of a single menstrual cycle. Four samples collected at one-week intervals were obtained from ten individuals and analyzed using SERS. Signal reproducibility was established by analyzing three gold nanoparticle chips for each sample solution and obtaining ten spectra per chip.

The SERS vaginal fluid signals showed very little variation as a function of time and storage conditions. The samples analyzed over the span of one menstrual cycle showed slight intra-donor differences; however, the overall spectral patterns remained consistent. When cycle spectra were compared between individuals, very little donor-to-donor variation was observed. A cross-validated, Partial Least Squares Discriminant Analysis (PLSDA) model was built to classify all body fluids, in which vaginal fluid was identified with 96.7% sensitivity and 99.6% specificity, which indicates that the spectral pattern of vaginal fluid was successfully distinguished from semen, blood, urine, and saliva.

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E40 Novel Azo Dye Presumptive Test for the Detection of Nitrites in Gunshot Residue (GSR): An Expansion of the Modified Griess Test

Erin M. Noval, BS, Cedar Crest College, 100 College Drive, Allentown, PA 18104; and Jeanne Berk, Cedar Crest College, 100 College Drive, Allentown, PA 18104*

After attending this presentation, attendees will better understand how the current modified Griess test can be altered to give greater contrast and improved detection of nitrites in GSR.

This presentation will impact the forensic science community by providing a more efficient and safer presumptive test for nitrites in GSR.

The current method detects the nitrites present in GSR by reacting them with sulfanilic acid, an aromatic amine, to form a diazonium ion. This then reacts with alpha-naphthol, an activated aromatic compound, to form an azo dye on the paper substrate. The paper substrate is prepared by soaking it in a solution of the reagents that are combined in equal parts and allowed to air dry. The item suspected of having GSR on it is processed by laying it over the dried paper substrate. Cheesecloth soaked in acetic acid is then laid over the top of the item and the stack is ironed without steam. If nitrites are present, an orange color dye will be visible on the paper substrate.[1]

Alpha naphthol was once thought to be carcinogenic, but now is listed as having unknown effects after chronic exposure and is considered toxic if inhaled. The new method presented uses reagents which are not suspected of causing cancer. Several reagents such as resorcinol and m-nitroaniline were tested in place of alpha-naphthol, and 1,3-metanilic acid and aminoanthracene were tested in place of sulfanilic acid. These changes resulted in a purple dye when reacted with nitrites.2 The limit of detection for Griess is 2.5µM.[3] The results show that using m-nitroaniline and aminoanthracene as the reagents detected nitrites at a concentration comparable to Griess, but was easier to discern due to its darker purple color. If the item being tested has blood on it, such as a suspected gunshot victim's shirt, a purple dye may be more visible through the blood, which would also be transferred during the reaction.

Traditionally, photographic paper that was desensitized was used as the reagent substrate, but due to decreased availability, laboratories are using photo paper, copy paper, both laser and inkjet paper, and standard laboratory filter paper more commonly. There is no standard from laboratory to laboratory.[4] This study conducted a survey of several paper types and found that high gloss photographic paper is the most durable after spraying with reagents and shows the most visual detail after being processed. Filter paper showed the greatest diffusion of the dye due to its porosity. The photo paper gave the best detail for the GSR pattern. Additionally, the reagent solution is generally applied by soaking the paper; by spraying the solution onto the paper with an aerosol sprayer, the solution is added uniformly and requires a lower volume. It also prevents warping of the paper caused by saturation.

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Griess Test, Nitrites, Azo Dye

E41 Multidisciplinary Study of a 17th-Century French Natural Mummy

Dedouit Fabrice, MD, Hopital Rangueil, Service de Medecine Legale, 1av. J. Poulhes, TSA 50032, Toulouse Ledex 9 31059, FRANCE; Fatima-Zohra Mokrane, MD, 1 Avenue Professeur Jean Poulhès, 31059 Toulouse Cedex 9, Toulouse 31059, FRANCE; Rozenn Colleter, Laboratory AMIS, 37 allées Jules Guesde, Toulouse 31073, FRANCE; Frederic Savall, Service de médecine légale, Hopital de Rangueil, 1 avenue Professeur Jean Poulhès, Toulouse Cedex 9 31059, FRANCE; Sylvie Duchesne, Laboratory AMIS, 37 allées Jules Guesde, Toulouse 31073, FRANCE; Patrice Gerard, Laboratory AMIS, 37 allées Jules Guesde, Toulouse 31073, FRANCE; Eric Crubezy, PhD, Université Paul Sabatier, 37 Allées Jules Guesdes, Toulouse 31000, FRANCE; Hervé Rousseau, PhD, 1 Avenue Professeur Jean Poulhès, Toulouse 31059, FRANCE; Daniel Rouge, MD, Service de Medecine Legale, Centre Hospitalier Univ Rangueil, Avenue du Professeur Jean Poulhes, Toulouse Cedex 4 31403, FRANCE; and Norbert Telmon, PhD, MD, Service Medico-Judiciare, CHU Rangueil, 1 Avenue Jean Poulhes, Toulouse F-31054, FRANCE*

The goal of this presentation is to illustrate the interests and the potentialities of the Multislice Computed Tomography (MSCT) in forensic anthropology and archaeology. A multidisciplinary study on a 17th-century French natural mummy presenting intentional heart removal will be presented.

This presentation will impact the forensic science community by providing an example of an original use of MSCT on a natural mummy.

Introduction and Background: In the Convent of the Jacobins (Rennes, Brittany, France, a rescue excavation was performed, permitting the study of approximately 1,000 graves dated between the 14th and the 18th centuries AD. In this collection, a perfectly preserved tomb was excavated in a 17th-century chapel. The body was that of a woman, dressed in a suit of probable Dominican community, buried in a trapezoidal lead sarcophagus. The body extracted from the lead sarcophagus was transferred in the Toulouse Hospital to undergo undressing, MSCT exploration, and autopsy.

The discovery of a well-preserved mummy thought to be a female noble was an exceptional opportunity for comparison between the findings of conventional autopsy and of MSCT before autopsy. This woman was born in 1584 and died in 1656. According to research, there has been no previous comparison of such data in a natural French mummy.

Material and Methods: A full-body MSCT was performed in Toulouse in the radiology department, after a complete undressing. This undressing permitted an external examination, revealing the presence of a cross-shaped, surgical thoraco-abdominal incision. The first hypothesis was that this incision represented an entry access for the embalming process. A complete autopsy was performed. Samples of tissues or macroscopic lesions were taken for complementary investigations, mainly bio-molecular analysis and ancient DNA determination.

Results: MSCT: (1) at the cephalic extremity, an aspect of intentional cranial deformation was visible. The rest of the brain was also visible. The bone structures were completely covered with a thick layer of a hyperdense material; (2) at the cervical stage, some carotid calcifications were visible. The spine was intact; (3) at chest level, the MSCT revealed that a thoracotomy had been realized, with a bilateral section of the sterno-costal cartilages. Furthermore, the pericardial sac was empty, with the heart absent. A mediastinal and pericardial cut was visible. It was possible to localize all the major thoracic vessels (arteries and veins) which were air filled. The lungs were present, presenting bilateral adherences; (4) at the abdomino-pelvic stage, some arterial calcifications were visible (aorta and internal iliac branches). Some hyperdensities were visible within both kidney parenchyma. The soft tissues of the posterior part of the body and the adjacent bones were hyperdense; and, (5) the bones appeared to be demineralized.

Autopsy — the autopsy confirmed all the MSCT findings: (1) the skull and most of the bones were black in color; (2) at the thoracic part, the section of the heart vessels was confirmed. Some ligatures were visible at the aortic and pulmonary artery trunk; (2) at the abdomino-pelvic stage, within both kidneys, some stones were found; and, (3) the organs were globally lytic, but with no evident major abnormalities.

Discussion: This case illustrates the complementarity of the PMCT and the autopsy for mummies.

In this case, the cause of death remained uncertain; however, some medical pre-existing pathological states were visible: calcified carotid plaques and voluminous kidney stones. Some taphonomical processes were visible: diffuse bone demineralization and hyperdense layer covering most of the bones (skull, lower limbs). This aspect of hyperdense layer was associated with dark bone coloration. According to research, this aspect has never been described. It is hypothesized that this aspect was due to a transfer of some metallic particles of lead, from the coffin to the soft tissues and the adjacent bones. Although the evidence of a surgical scar first oriented toward an artificial mummification process, the mummy was finally determined to be a natural mummy. The MSCT revealed the heart evisceration, with a bilateral cut of sterno-costal cartilages. The rest of the exploration revealed no other organ removal.

Conclusion: This case demonstrates that MSCT and post-processing techniques are indispensable tools in the multidisciplinary investigation of mummies.

Natural Mummy, Multislice Computed Tomography, Autopsy

E42 K9 Water Searches and Volatile Organic Compounds (VOCs): A Method to Aid in Determining the Location of Submerged Human Bodies

Marcello Rendine, Viale degli Aviatori 1, Foggia 71100, ITALY; Cristoforo Pomara, MD, PhD, University of Foggia, Dept Forensic Path, University of Malta, Dept of Anatomy, Faculty of Med & Surg Biomedical Sci, Foggia, Misida, Malta 71100, ITALY; Carmela Fiore, MD, Ospedale Colonnello D'Avanzo, Viale degli Aviatori 1, Foggia 71100, ITALY; Palmira Fortarezza, MS, Ospedale Tatarella, Cerignola, ITALY; Francesco Sessa, MS, Ospedale Colonnello D'avanzo, Viale Degli Aviatori 1, Foggia 71100, ITALY; and Irene Riezzo, MD, PhD, University of Foggia, Osp D'Avanzo, Dept of Forensic Pathology, Viale degli Aviatori, 1, Foggia 71100, ITALY*

The goal of this presentation is to demonstrate to the forensic science community specific VOCs from submerged human bodies that elicit an appropriate response by recovery canines (K9s).

This presentation will impact the forensic science community by showing how the identification of the VOCs released by submerged pieces of organs may be useful to better define VOCs produced by human material decomposition and as aids for training water search cadaver dogs to identify submerged human bodies.

Forensic personnel are frequently requested to locate submerged victims of homicides, drownings from boating accidents, or suicides. Search methods used to locate submerged bodies routinely include deploying underwater cameras or specialized dive teams, though properly trained cadaver dogs can also be effective at locating submerged human bodies or remains.

A recent increase in the use of trained water search canines for detecting submerged human bodies has created the need to have an exact knowledge and awareness of the volatile chemical signature of compounds that could indicate the presence of submerged human bodies. Although human scent is defined as the most abundant VOC, only a few VOCs, emanating from the submerged bodies, transit the water to stimulate canine olfactive alerts. Indeed, dogs don't smell submerged bodies through the water. VOCs from submerged bodies can enter the water, rise through it to the surface, and so enter the air to be detected by the canine olfactory system.

This study to detect the VOCs released from submerged human cadaveric bodies, which stimulate canine olfactive alerts, was performed using Gas Chromatography/Mass Spectrometry (GC/MS). Pieces of organs (skin, muscle, fat, brain, heart, lung, spleen, liver, and kidney) from four traffic-accident fatalities (two men and two women, excluding cases of intoxication) were used. The samples were stored in 24 separate glass jars (12 containing salt water and 12 containing fresh water). The glass jars were covered by a film with holes in it, above which were arranged several VOC-free cotton gauze pads, then the jars were closed by a cover. The water temperature at the surface was 0°C (4 salt water and 4 fresh water), 15°C (4 salt water and 4 fresh water), and 30°C (4 salt water and 4 fresh water). The gauze was used in part for the chemical analysis and in part for dog training procedures.

The first extraction was assessed on the gauze as time 0 of the experiment. The headspace extractions were repeated every 6 days for 120 days for each glass jar (20 extractions for each glass jar). The National Institute of Standards and Technology (NIST) mass spectral library and extracted ion chromatograms were used to identify the compounds.

More than 100 VOCs have been identified. Only VOCs that have been previously cited in the literature as originating from human specimens were used in the analysis of these samples as key markers of the presence of submerged bodies, from the water to the surface. The various molecules so identified, assessing their changes according to the temperature of the water, the decomposition process, and the water salinity were analyzed and selected. These results were included in a canine's training program in order to improve it to support the ability of using olfaction to locate submerged human bodies.

The results of this study indicate that the well-trained water search dog is an outstanding tool for detecting submerged human bodies, displaying excellent sensitivity (between 99.42% and 100%), having a Positive Predictive Value (PPV) ranging between 94.97% and 100%, and a Negative Predictive Value (NPV) ranging between 85.71% and 100%.

These recovery rates ranging between 99% and 100% indicate that properly trained water search dogs can make significant contributions in the location and recovery of submerged human bodies.

K9 Water Searches, Submerged Human Victims, Volatile Organic Compounds

E43 Death From Hypothermia During a Training Course Under "Extreme Conditions": Two Related Cases

Lucile Tuchtan, MD, 63 Chemin Des Aurengues, Marseille 13013, FRANCE; Pierre Perich, MD, Department Forensic Sciences, 264 Rue Saint Pierre, Marseille 13013, FRANCE; Georges Leonetti, PhD, 264 Rue Saint Pierre, Marseille, FRANCE; Marie-Dominique Piercecchi-Marti, PhD, 264 Rue Saint Pierre, Marseille 13005, FRANCE; and Christophe Bartoli, MD, 264 Rue Saint Pierre, Marseille, FRANCE*

After attending this presentation, attendees will understand that in typical and clinically confirmed cases of death from sub-acute exhaustion hypothermia in subjects with an optimal natural defense system, none of the signs observed in the autopsy (flaccidness and coloring of the skin, cerebral and pulmonary edema, pink markings on the lungs) are specific. Although some recent publications have addressed the utility of postmortem biochemical markers when establishing a diagnosis with no anamnesis, no knowledge or analysis of the circumstances of death, and without an *in situ* examination of the body, it appears difficult, if not impossible, to confirm that death was caused by hypothermia.

This presentation will impact the forensic science community by the relevance of these typical cases. Comparing typical hypothermia cases of dead and living subjects demonstrates that, despite the advances in research on biochemical markers, these will remain non-specific.

Death from hypothermia following exhaustion or from various complicated pathologies is no longer a frequent cause of death among combat troops. During a training course under "extreme conditions" in the French Alps, two young African officers died. Confronted with these two clinically confirmed cases of hypothermia, the unknown anatomopathological and biological specificities associated with death from hypothermia were highlighted. In these typical and clinically confirmed cases of death from sub-acute exhaustion hypothermia, none of the signs revealed by the autopsy were specific.[1-3]

During the autopsy, the following were noted in both subjects: congestive and edematous brain tissue, pulmonary edema with large pink lesions on the anterior border of the lungs associated with a small number of darker lenticular lesions, congestive abdominal organs, and a full bladder. Based on the microscopic examination, the following was observed: non-specific, anoxic cerebral lesions; alveolar edema with intra-alveolar hemorrhagic alterations; non-specific gastritis lesions; and blood in the peripancreatic fat with no other architectural modifications of the gland that could have resulted from resuscitation maneuvers.[3]

Overall, the necroscopic signs were scarce and completely lacking in specificity.

Finally, a substantial increase in thyroid hormones with a concomitantly and paradoxically clear increase in the Thyroid-Stimulating Hormone (TSH) content was observed, which is indicative of an immediate and total involvement of the hypothalamo-pituitary and thyroid axis at the outset of the adverse weather conditions, to support the "first-line" pituitary hormones.[4-8]

At the biological level, the total urinary catecholamines remained at normal levels (1.2 and 1.5µmol/24 h (0.8-2.1)), whereas their derivatives had significantly lower values than normal (0.7 and 1µmol/24 h (2.1-4.2µmol/24 h)). Major hypoglycemia (1.2 and 1.3mmol/l (4.2-6.6 mmol/l)), a highly significant increase in transaminases (ALAT at 738 and 1012UI/l (8-65 UI/l)), and an increase in serum creatinine (160 and 135µmol/l (62-106 µmol/l)) were also found.

In the literature, it is observed that urinary catecholamines, free fatty acids of the blood, blood corticosteroids (especially cortisol), and free urinary cortisol can increase following death from hypothermia, independent of blood ethanol concentrations. Although this biological data may confirm the diagnosis of death from hypothermia, it is important to emphasize that the quality of conservation of the biological specimen and the time between sampling and analysis will have a significant influence on the stability of urinary catecholamines. Thus, normal levels of urinary adrenalin in such cases of suspected death from hypothermia do not allow such a diagnosis to be excluded.[9-13] Similarly, an increase in the concentration of free fatty acids and corticosteroids in the blood cannot be used as the sole criterion for the diagnosis of fatal hypothermia; however, an increase or decrease in corticosteroid concentrations in the blood and urine can also be a symptom of pre-existing diseases, leading to the conclusion that glucocorticoids, just as other biochemical parameters, can be considered potential markers of fatal hypothermia when all of the other postmortem results are taken into account.[9-13]

In conclusion, although some recent publications have addressed the utility of postmortem biochemical markers when establishing a diagnosis, with no anamnesis, with no knowledge or analysis of the circumstances of death, and without an *in situ* examination of the body, it appears difficult, if not impossible, to confirm that death was caused by hypothermia.

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Hypothermia Death, Combat Troops, Biochemical Markers

E44 Use of Infrared Photography to Document Bloodstains in Fire Scenes

William K. Perdue, MPA, Bureau of Alcohol, Tobacco, Firearms and Explosive, 2600 Century Center, Ste 300, Atlanta, GA 30345; Elizabeth Richards, PhD, Defense Forensic Science Center, 4930 N 31st Street, Bldg 925, Forest Park, GA 30297; and Maria C. Castellanos, MFS*, Air Force Office of Investigations, 4930 N 31st Street, Bldg 925, Forest Park, GA 30297*

After attending this presentation, attendees will understand the effectiveness of Infrared (IR) photography when used to detect, visualize, and document bloodstains in fire scenes.

This presentation will impact the forensic science community by providing a comparison of the effectiveness of two non-destructive techniques (white light and IR photography) when employed to visualize and document bloodstain patterns in crime scenes where fire artifacts such as soot obscure valuable evidentiary material.

Soot deposition and other fire-related artifacts can obscure bloodstains to the point where they are difficult to identify with the naked eye. Due to the obstruction, additional methods of locating and documenting bloodstains need to be evaluated.

It is often difficult to detect bloodstain patterns on dark surfaces due to the lack of contrast. IR photography has been used to detect bloodstains on dark surfaces (fabrics) because infrared light (700nm-1,500nm) is absorbed by blood, providing contrast between the blood and the background surface; however, there are no reports in the literature on the use of IR photography to detect bloodstains covered by soot in fire scenes. This study evaluated and compared the ability of both white light and IR photography to visualize soot-covered bloodstains.

This research was conducted at the Alcohol, Tobacco and Firearms National Academy located on the Federal Law Enforcement Training Center, GA. Gypsum board (drywall) was installed on the inside of each of three concrete burn cells. This transformed each burn cell into a 9' x 13' room with an 8' ceiling. Therefore, each burn cell was both the crime scene and the room of fire origin. The following surfaces were utilized on each 13' wall in each burn cell: dark gray paint, white paint, bare gypsum board, laminate flooring, and patterned wallpaper. Each 13' wall was divided into upper and lower sections 4' off the floor. Two volunteers donated blood via venipuncture. Cast-off, transfer, and impact spatter bloodstains were deposited on each surface type in both the upper and lower sections for a total of 30 bloodstains/wall and 60 bloodstains/cell. All bloodstains were then photographed using white light photography. TRUFuel Engineered Fuel and Oil® products were used as the accelerants and the fires were suppressed using water. Each cell burned between two and two and one-half minutes at temperatures ranging between approximately 800°C and 900°C. After each burned cell cooled to safe temperatures, photographs of the bloodstains were accomplished with white light photography. Next, all bloodstains were digitally captured using a Foster+Freeman Crime-lite® 82S IR Forensic Light Source with an attached IR-sensitive camera which displayed live images on a computer. Lastly, representative bloodstains were photographed using a full-spectrum modified Nikon® D300 equipped with a Nikkor 60mm micro lens and Peca® 904 IR band pass filter.

Post-fire, all but one bloodstain was detected with the naked eye, oblique white light, Crime-lite®, white light photography, and IR photography. The blood stains were covered with soot but not completely obscured. The visible stains deposited on the light surfaces (white paint and gypsum board) were more easily identified than those deposited on the dark gray paint. In both the white light and IR photographs, the bloodstains appeared darker than the background surface (despite the color). Both the wallpaper and laminate flooring (secured to each wall) were significantly altered due to the high temperatures. Only a few blood drops were visible on the remnants of the laminate flooring and it was not possible with either type of photography to determine from which type of stain the drops originated. On the wallpaper, it was not possible to determine the location of the blood. While there were areas on the remnants of the wallpaper that may have been blood, neither type of photography allowed a conclusive determination.

The results of this study show that white light and IR photography performed equally well when documenting bloodstains after fire when the stains were still visible to the naked eye. Under the conditions of this study, IR photography provided the same level of detail for bloodstains already identified by white light photography. In future studies, it would be beneficial to further explore the effectiveness of IR photography to detect bloodstains when they are completely obscured by soot.

Infrared Photography, Bloodstains, Fire Scene

E45 The Relevance of a Multidisciplinary Approach to the Crime Scene Investigation: A Case Report of a Homicide Victim Who Was Hidden

Natascha Pascale, MD, Viale Degli Aviatori, Foggia 71100, ITALY; Marcello Rendine, Viale degli Aviatori 1, Foggia 71100, ITALY; Francesco Sessa, MS, Ospedale Colonnello D'avanzo, Viale Degli Aviatori 1, Foggia 71100, ITALY; Dania De Carlo, MD, Ospedale Colonnello D'Avanzo, Viale degli Aviatori 1, Foggia 71100, ITALY; and Irene Riezzo, MD, PhD, University of Foggia, Osp D'Avanzo, Dept of Forensic Pathology, Viale degli Aviatori, 1, Foggia 71100, ITALY*

The goal of this presentation is to emphasize the validity of evidence from crime scene investigations through the cooperation of the forensic pathologist, the cadaver dog team (dogs trained to locate human cadaveric blood in very low concentrations and cadaver water search dogs that detect submerged human bodies), and the use of forensic field tests which allow the detection of latent human traces, providing key evidence to solve complex crime cases.

This presentation will impact the forensic science community by showing how proper crime scene processing and collection of evidence provide the preliminary basis for any subsequent forensic investigations, testing, and analysis.

This case report concerns the involvement of a forensic team in police investigations of a woman who had already been missing for four months. The 38-year-old foreign national lived in an isolated country house. The police extensively searched an approximately 50-mile area surrounding the woman's house.

The forensic team consisted of the forensic pathologist and the canine unit (a 7-year-old male Labrador retriever) trained for the detection of latent traces of cadaveric blood. In addition, the use of a water search dog (a 3-year-old male Labrador retriever) enabled the team to extend the search into a lake. Starting from the home of the missing woman, the track followed by the dogs led and stopped at a covered well, 20 miles away from the woman's house, in an uninhabited area. On the ground surrounding the well, latent traces of blood were detected by the dogs. The latent traces were confirmed by the luminescence of the crime light, noticeable by daylight, and a prompt test with a latent bloodstain luminal reagent was performed. The advantage of this reagent consists of the ability of the heme to catalyze the chemo-luminescence property of luminol; it will emit light, which can be noticed in the dark. Additionally, the proof that the latent traces contained human hemoglobin was delivered by an immunochromatographic rapid test, which is based on the reaction with monoclonal anti-human Hb antibodies.

Inside the well, a corpse was discovered floating in the water. The external examination and a complete autopsy revealed a decomposing body of a woman murdered by multiple rifle shots to the neck and abdomen. The subsequent DNA analysis showed that the woman was the same person that had gone missing four months earlier and that the traces of human blood detected during the investigation belonged to the same woman. The forensic team was then asked to survey the country house and the van owned by a farmer who was stopped by a police officer in connection with the murder of the woman. Using the same method described above, it was possible to identify other latent traces of human blood, which later DNA testing proved to be that of the murdered woman. With this proof, the police obtained the confession of the woman's murder by the farmer.

Events leave physical traces which constitute a physical evidence record of the event.

This case report demonstrates how an interdisciplinary approach provided investigators with the tools necessary to succeed in solving the case, even when starting from meager elements of investigation. A proper crime scene processing and collection of evidence, an appropriate use of a field test, the incorporation of a well-trained cadaver dog and water search dog allowed investigators to suitably evaluate what happened, recognize the subject of the investigation, and ultimately secure a confession and subsequent conviction of the perpetrator. The data derived from a successful crime scene investigation provide the preliminary basis for any subsequent forensic investigations, testing, and analysis.

Essentially, the proper crime scene investigation establishes the evidence, both criminal and scientific, on which the entire investigation framework is based.

Crime Scene Investigation, Field Test, Cadaver Dog

E46 Sudden Unexpected Infant Deaths (SUID) in North Central Indiana — A Comprehensive Look at Infant Deaths in Indiana

Matthew C. Wietbrock, BS, 629 N 6th Street, Lafayette, IN 47909*

After attending this presentation, attendees will better understand the frequency of SUID deaths in Tippecanoe County, IN, from 2010 to 2015. Attendees will also be informed as to circumstances of these deaths, including decedent's age, medical history, postmortem autopsy results, toxicology results, and other factors, which were considered to have contributed to the death.

This presentation will impact the forensic science community by showing how the mystery of SUID continues to be a perplexing issue for the forensic medical and death investigator community. In addition, the topic of co-sleeping, or bed-sharing, has become a "hot-button" issue in recent years. Advocates for and against this practice are passionate in the apparent benefits and dangers associated with sleeping with an infant. Numerous tragic cases of such deaths have occurred in the Tippecanoe County community in recent years.

Added to this conflict is a category of SUID, the phenomenon of Sudden Infant Death Syndrome (SIDS), in which seemingly healthy infants die and, through a process of exclusion, are labeled as SIDS deaths.

Tippecanoe County is located in north central Indiana, 124 miles southeast of Chicago, and 68 miles northwest of Indianapolis. It is home to Lafayette, IN, and West Lafayette, IN, numerous manufacturing industries, and a major research university. This wide range of opportunity allows for a very diverse population of more than 177,000. This community has experienced numerous SUID deaths since 2010.

The results of these death investigations will be presented, including: demographics of the deceased, demographics of the family involved, recent medical history of the deceased, and autopsy results. The type of SUID death (i.e., SIDS, Unknown, or Accidental Suffocation and Strangulation in bed) will be displayed. If co-sleeping was found to be a possible contributing factor, the relationship of the person sleeping with the infant at the time of death will also be discussed. The decision toward, and challenges to, any subsequent criminal charges will also be disclosed.

The location of the event will also be identified, such as in a bed, couch, chair, etc. Recent data suggests that co-sleeping on a couch, or even just outside of a bed, is even more dangerous. Any indication of child abuse, family history of SIDS, or other infant sleeping deaths within the family will also be of interest.

The heartbreaking nature of these deaths leaves many questions yet to be answered. The goal of this presentation is to foster awareness among the forensic community, in the hopes that trends, if any, can be identified.

SUID Death, SIDS, Investigation

E47 The Impact of Fentanyl Use and Abuse

Breanna M. Cuchara, 31 McDermott Street, Milford, CT 06460; and Thomas A. Andrew, MD, OCME, 246 Pleasant Street, Ste 218, Concord, NH 03301*

After attending this presentation, attendees will understand the impact pharmaceutical-grade fentanyl and its synthetic analogues being sold on the street have on individuals. The most common sources of the latter agents are also discussed.

This presentation will impact the forensic science community by showing the potential dangers of fentanyl both in a medical setting as well as a street drug of abuse, particularly recreational drug users whose drug of choice has been heroin.

Fentanyl is a potent, synthetic opioid analgesic that acts quickly and has a long duration. Unlike heroin, fentanyl can be used in a pharmaceutical setting and in an illegal setting. When produced legally, it is made in a pharmaceutical laboratory under a license.[1] In a pharmaceutical setting, fentanyl can be used for surgical anesthesia and pain relief.[1] It can be used to treat pain in cancer patients as well as chronic, unremitting, non-cancer pain. Transdermal fentanyl patches may be prescribed for this purpose. This patch can lead to an accidental overdose or death due to its long-lasting effects even when used correctly. Additionally, the patch has the potential of being misused.[2]

An example of misuse is a case researched by reading the police report, toxicology report, and the medical examiner's report, as well as through discussion with the medical examiner. In this case, a mother put her prescription fentanyl patch on her crying child, who was younger than 12 months old. This led to the child's death by acute fentanyl intoxication.

Fentanyl is typically prescribed in microgram amounts due to its potency.[3] When combined with other drugs such as heroin, the likelihood of fatal intoxication is enhanced due to fentanyl being much more potent than heroin, with both having the capacity for profound respiratory depression.3

Fentanyl is also used illegally and sold on the black market. Used alone or when combined with other agents, particularly opiates/opioids, it can easily bring about fatal respiratory depression, similar to morphine or heroin.[4] In this medicolegal jurisdiction, many users have purchased what they may have believed was heroin that had been combined with fentanyl or an even more potent fentanyl analogue and its use has caused death.

Fentanyl has a chemical structure that can be easily manipulated to synthesize variations of the drug.[4] Many analogues are sold on the street and are known as either "synthetic heroin" or "China White."[4] Many users are led to believe that they are buying heroin, but the drug is actually fentanyl or one of its analogues. More than 12 different analogues of fentanyl are being produced illegally, most commonly in Mexico.[5] Across the nation, overdose deaths from fentanyl or from heroin/fentanyl combinations have increased tremendously.[3]

Although fentanyl is very important for pain relief, it also has enormous potential to be abused by both the persons to whom it has been prescribed and by people using it illegally. In the presented case, it was believed to have been improperly used to sooth a young child. The mother's explanation of the incident was that the patch accidentally migrated from her body to her infant during a nap together. Regardless of the specific mechanism by which the patch came to be on the infant, the case further demonstrates the risk of unintentional fatal intoxication due to ease of use (transdermal patch) and its long duration of action.

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Fentanyl, Painkillers, Drug Abuse

E48 "All the Things You Ask of Me": Law Enforcement Experiences of Infant Death Investigation

Jennifer R. Schindell, MA, 34296 Kamph Drive, NE, Albany, OR 97322*

After attending this presentation, attendees will better understand law enforcement experiences and perspectives related to infant death investigation.

This presentation will impact the forensic science community by contributing data to an aspect of death investigation for which scant previous research has been reported. This presentation will also reveal findings germane to efforts targeted at improving infant death investigation practice and outcomes.

The sudden and unexpected death of a seemingly healthy infant sets in motion a number of linked processes with potentially complex and far-reaching ramifications. While individuals, families, and communities grapple with the shock and heartbreak associated with the loss of a young life, a chain of multidisciplinary investigative responsibilities is initiated to address the question of causation. Currently, very little is known about how infant death investigations are conducted and, perhaps more importantly, how variability within structures and processes influence individual or aggregate case outcomes. Specifically, very little attention has been paid to the role of law enforcement within either the larger medicolegal death investigation structure or in the specific realm of infant death investigation. Though the medical examiner or coroner bears ultimate responsibility of certifying the cause and manner for a Sudden Unexpected Infant Death (SUID), medicolegal death investigations unfold as multidisciplinary endeavors dependent upon information obtained from a variety of sources; law enforcement officials invariably play a significant role in this process. In smaller and/or poorly funded jurisdictions, law enforcement may serve as the sole investigators of death; however, even when designated medicolegal death investigators participate on behalf of the coroner or medical examiner, law enforcement officials can still contribute significantly to a case. Patrol officers and deputies are often among the first to arrive at any death scene and, therefore, have the potential to significantly influence the trajectory of any investigation. Additionally, law enforcement investigators bear the burden of any resultant criminal investigation.

The purpose of this study was to examine the experiences of law enforcement officials charged with investigating SUIDs in nine Pacific Northwest jurisdictions. Data collected from open-ended, semi-structured interviews (n=26) revealed three dynamic and interrelated tensions experienced by law enforcement during the process of infant death investigation. Furthermore, their stories reveal that occupational norms and values, which may serve them well in many other circumstances, falter in the context of infant death investigations. Fortunately, participants' narratives also provided a foundation upon which to build recommendations intended to improve the efficacy of investigations, while simultaneously reducing tensions experienced by investigators and others involved in the challenging scenario of an SUID.

Infant Death, Law Enforcement, Investigation

E49 Case Study: Perfect Crime? The Forensic Sciences at the Service of the Crime

Eric R. Ruiz Hernandez, MD, Calle 23D 86 28, Torre 6 Apto 303, Bogota, Cundinamarca 110911, COLOMBIA*

After attending this presentation, attendees will understand how, through a comprehensive process of analysis of the evidence at the crime scene, a reconstruction of a criminal behavior was obtained in which a police colonel, an expert in crime scene investigations, tried to hide the driving motivation and the dynamics of the murder of his wife.

This presentation will impact the forensic science community by showing how, despite being an expert in the processing of crime scenes, fingerprinting, and forensic anthropology, a police officer tried to manipulate and alter the evidence. Thanks to the proficient processing of the crime scene and an adequate interpretation of the behavioral evidence by the investigative and criminal profilers teams, the real dynamics of the offense and what the criminal's motivation was is explained.

From September 9-12, 2009, segments of a human body were discovered in different fields of crops and road sides leading from the town of Ibague to other municipalities in the state of Tolima, 160 miles from Bogota D.C., the capital of Colombia.

The autopsy identified the victim as a woman who died as a result of direct trauma with a blunt item that fractured her skull and was dismembered postmortem. The murderer disfigured the victim's face with 58 cuts, removed her finger pads, extracted her third and fourth left ribs and her breast implants, and mutilated her genitals. The body was identified as the wife of an important police officer in this state, reported missing by her family.

The characteristics of the murder of this police officer's wife activated the police's special investigative units in the country, thus the Criminal Behavior Analysis Group of the Attorney General's Office was called upon to support the investigation through a process of criminal behavioral analysis and the possible developing of a criminal profile.

Based on the investigation of the crime scene, specifically the analysis of the basement of the house the couple shared with their daughters, the discovery of blood spatter patterns found by the application of a chemiluminescence reagent, the recovery of trace evidence (specifically, human blood which matched the victim's genetic profile), the victimology, and the evaluation of the versions provided by the police colonel and other witnesses, the team was able to redirect the investigation and establish that the crime was committed and motivated by passion.

This new view in the case offered the team a clear vision about the dynamics of the events that led to the death of the woman. Therefore, the line of investigation focused on probing deeper into the couple's relationships. Discovered was an intricate world of infidelity and a husband with a passive-aggressive personality, who protected his professional and social reputation and did not accept his wife's intention of leaving him.

After fitting all parts of this criminal and forensic puzzle together, the prosecutors demonstrated definitively and beyond reasonable doubt that the crime was committed by the colonel, who was sentenced to 43 years in prison; on appeal, the sentence was upheld by the superior court.

Crime Scene Investigation, Criminal Behavior Analysis, Victimology

E50 Retrospective Analysis of 93 Male Victims of Unnatural Sexual Offenses From 2011 to 2014 in a Tertiary Care Center in India

Shashank Pooniya, MD, AIIMS, Room No. 6, Mortuary, AIIMS, Ansari Nagar, New Delhi, Delhi 110029, INDIA; Rajanikanta Swain, MD, All India Institute of Medical Sciences, Rm No-93, Hostel N0-8, AIIMS, Ansari Nagar, New Delhi, Delhi 110029, INDIA; and Sanjeev Lalwani, MD, Department of Forensic Medicine, AIIMS, New Delhi 110029, INDIA*

After attending this presentation, attendees will better understand how male-on-male sexual assault cases are dealt with by forensic experts in India. This presentation will also discuss the socio-demographic features and major physical findings related to the victims of male-on-male sexual offenses.

This presentation will impact the forensic science community by providing information about the current scenario of male-on-male sexual offenses in a developing country such as India. It will also focus on the examination of victims of male-on-male sexual assault, common injury pattern, evidence preserved, and the details to be noted in medicolegal case reports.

The long-term effects of sexual abuse of women by men have been studied extensively, but there has been minimal research exploring sexual assault by men on other men. In fact, there are very few studies regarding male rape. In India, the situation is complicated by the fact that, according to 375 and 376 Indian Penal Code (IPC), rape can only be performed by a male on a female and the crime related to sexual assault on a man is included under a separate portion of the IPC (IPC 377). Personal stories of male rape mirror female rape in terms of a sense of shame, humiliation, and self-blame, but males are even less likely than females to report an assault.[1] Fear of being labeled a homosexual and society's concept that a "real man" cannot be raped may lead to a significant under-reporting of cases.[2,3]

The medicolegal case reports of 93 victims of rape were reviewed. The men were brought in for a medical checkup to the Department of Forensic Medicine in a tertiary care center of India over a period of four years (January 2011 to December 2014). The mean age of the victims was 12.5 years. The greatest number of the victims were 11 years to 15 years of age (36.6%), followed by the 6-year to 10-year-old age group (33.3%). Out of all 93 victims, 81 were juveniles 0 years to 18 year of age (87% of the total number of victims). Only 12 victims (13%) were adult (>18 years of age). In 83 cases, sexual assault was solely sodomy, while two cases were solely buccal coitus. Eight cases involved both sodomy and buccal coitus. The most common sign/ symptom was local pain and tenderness in the anal region (28 cases), followed by localized injury in the anal region (18 cases). Only 12 cases had associated bodily injuries. Among the 18 cases with anal injury, the most common position of the injury was 12 o'clock (nine cases, 50%) followed by 6 o'clock (eight cases, 45%). The most common evidence collected was blood in gauze for DNA analysis, collected in all 93 cases. Anal and perianal swabs were collected in 81 cases (87%) and undergarments were collected in 70 cases (75%).

In conclusion, this study provides an insight into the existing scenario of male-on-male sexual assault, which is often neglected or given less value than would be a sexual assault on a female. Furthermore, data from this study helps in identifying the common injury pattern and evidence to be collected. It will also help in identifying the "do's" and "don'ts" during the medicolegal reporting of the victim of male-on-male sexual assault.

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Unnatural Sexual Offence, Male Rape, Sexual Assault

E51 Shaken Baby Syndrome/Abusive Head Trauma (SBS/AHT) Mortality in Sweden

Jacob Andersson, MS, Uppsala University, Västerlånggatan 45, Eksjö, Småland 75232, SWEDEN; and Ingemar Thiblin, PhD, Uppsala University, Rättsmedicinalverkets Rättsmedicinska Avdelning, Box 1024, Uppsala 75140, SWEDEN*

After attending this presentation, attendees will better understand data of the mortality and specifics of cases of suspected SBS/AHT in Sweden. This is the first study on SBS/AHT based on national population registries.

This presentation will impact the forensic science community by illustrating a far lower mortality rate than expected from previous studies in other western countries together with a high prevalence of various diseases in deceased infants diagnosed with AHT. The present study underlines the need for further epidemiological mortality research and therefore a cautiousness in relying on currently established mortality data.

Objective: On the basis of extrapolation from international studies on mortality related to SBS or infant AHT, it has been suggested that the incidence of lethal SBS/AHT as reported in the Swedish official death statistics is falsely low. The present study investigates the possibility of unrecognized lethal AHT in Sweden.

Design: All death certificates of infants 12-months-old or younger (*n*=733) examined at any of the six Swedish departments of forensic pathology during the period 1994-2013 were retrospectively reviewed for identifying subdural hematoma or an SBS/AHT diagnosis. In order to exclude possible misclassification of SDH related to accidental impact or disease/developmental vulnerability as SBS/AHT, confessed abuse, evidence of blunt force/impact, disease, prematurity, and multiparity were registered. The possibility of non-reported subdural hematoma in infant deaths was evaluated by scrutinizing the autopsy protocols for all infants diagnosed with unknown cause of death during 2006-2013 (*n*=167).

Results: Of the 733 forensically examined cases, fourteen (1.9 %) had SDH. Four were witnessed accidents, one infant had been thrown from great height, and nine were considered as SBS/AHT. Seven AHT-classified infants were premature and/or twins and/or had significant morbidity. In one case, the caretaker confessed to AHT; the infant in this case had findings consistent with impact. There was no case of non-reported subdural hematoma in the group with unknown cause of death. National statistics on cause of death included 89 cases diagnosed as death from an unknown cause in which the infant did not die within the first week of life and which had not been reviewed by a forensic pathologist. This group could not be examined in this study setting and needs to be reviewed in the future.

Conclusion: The present study does not support the notion that there might be unrecognized lethal AHT in Sweden, but rather suggests the opposite — that there may have been infant deaths wrongly diagnosed as caused by AHT.

Abusive Head Trauma, Shaken Baby Syndrome, Sweden

E52 Case Study: From Maternal Instinct to Staged Domestic Homicide

Eric R. Ruiz Hernandez, MD, Calle 23D 86 28, Torre 6 Apto 303, Bogota, Cundinamarca 110911, COLOMBIA*

After attending this presentation, attendees will understand how, through a process of comprehensive analysis of the crime scene, a reconstruction of a criminal behavior was obtained that was the driving motivation of a murder against two children which was perpetrated by their own mother.

This presentation will impact the forensic science community by describing how the criminal conduct of a staged domestic homicide has some repetitive patterns or common characteristics in terms of planning and execution, regardless of where in the world it occurs, without being influenced by sociocultural or economic factors of their executors.

A mother reported to the Mocoa police (Mocoa is the capital of the department of Putumayo in southern Colombia) that in the early hours of September 20, 2010, an unknown individual attacked her when she opened the front door of her residence. She was attacked until she lost consciousness. After regaining consciousness, she found her children in bed in the master bedroom. When approaching them, believing them to be asleep, she noted they were wet and without signs of life.

The initial investigation found that the woman's injuries were consistent with blunt trauma. The apparent coherence of her story led the authorities to think there was a perpetrator who had murdered the two children and left their mother seriously injured. The high social impact produced by a case of two murdered children and the assault on their mother activated both local and national law enforcement, resulting in the Criminal Behavior Special Unit of the Attorney General's Office being called in to support the investigation through a process of crime scene analysis and the possibility of developing a criminal profile.

On the basis of the crime scene analysis, the patterns of blood stains found, the comprehensive study of the evidence related to the victims, the assessment of the versions provided by the surviving mother and other witnesses, and the structural characteristics and safety of the building, the investigation team re-evaluated the hypothesis of an external aggressor and redirected their attention toward the mother.

This change in the case offered the team a clear vision about the dynamics of the events that led to the deaths of the two children. With the support of the analysis of forensic evidence at the scene, the Criminal Behavior Special Unit was forced to focus their attention on finding the motivation that drove this woman to kill her own children. The investigation focused on probing deeper into all areas of the mother's life and found an intricate world of love frustrations and abandonment, a mentally unhealthy woman, egocentric and possessive, with strong intentions to overcome any obstacle to achieve all personal desires.

After coordinating and fitting all parts of this criminal and forensic puzzle together, the prosecutors demonstrated definitively and beyond a reasonable doubt that the crime was committed by the mother in the form of a staged domestic homicide.

Mother's Killer, Children, Staged Domestic Homicide

E53 Child Abduction Murder: Regional Differences in Time to Death and Offender Motivation

Katherine M. Brown, PhD, Tarleton State University, Dept of Criminal Justice, 6777 Camp Bowie Boulevard, Ste 500, Fort Worth, TX 76116*

After attending this presentation, attendees will better understand the differences in child abduction murders across the United States. Information from United States regions and states will be the focus of this presentation.

This presentation will impact the forensic science community by adding geographically specific information to an under-researched area, child abduction murder investigations. Specifically, this presentation will identify geographic differences in how long children were kept alive after abduction and offender motivation. This presentation will also provide investigators with a better idea of how long different age categories of children were kept alive after they were abducted as well as differences in time to death by gender and geographic location.

There are relatively few empirical studies on child abduction murder.[1-4] In particular, little research has addressed the influence of time and distance on case solvability in murder investigations of abducted children.[1,2] To date, only one study has addressed the impact of forensic evidence on child abduction murder investigation solvability.[5] No studies have provided detailed information on child abduction murders by exploring geographic location differences.

The geographic differences in child abduction murders were explored by examining child abduction murders occurring from 1968 to 2002 (N=735) across the United States. The following geographic locations are the primary focus of this presentation: Texas, Washington State, Wisconsin, and the Northeast Region (consisting of New England and Mid-Atlantic States). Geographic differences were explored by analyzing the time between when the child was abducted and when the child was killed. Differences were also examined by victim gender and age-group category.

An added challenge to child abduction investigations is that there typically is a time lapse between when a child goes missing and when that child is reported missing to police. While approximately 40% of children were reported missing within two hours, the remaining 60% were not.[3] Children are killed quickly after abduction in child abduction murders. Prior analyses indicate that as the victim's age increases, the time span between when the victim is abducted and when the murder occurs does not always increase. Abducted children who were murdered were typically killed within three hours (76.2%).[1-4] Young children between the ages of 0 years to 5 years old were killed within three hours at a higher percentage than the other age groups (81.8%, $p > .05$). Young children (0 years to 5 years old) were killed more quickly than middle childhood victims (6 years to 11 years old), young teenagers (12 years to 14 years), and older teenagers (15 years to 17 years); however, there are distinct geographic differences in how long children in each age-group category are kept alive after an abduction.

Analyses by geographic location indicate that offenders may also have differing motivations by region. For instance, killers in the Wisconsin region committed a higher percentage of sexual assault on their victims than other regions. Geographic-specific findings provide valuable information to investigators in the absence of other leads or evidence. Most child abduction murders are crimes of opportunity; therefore, knowledge about regional differences in offender motivation will provide valuable investigative direction in the absence of other leads. In addition, differences in the time an abductor keeps a victim alive by age group and gender may provide insight into region-specific investigative challenges. This examination adds to the literature on how time and distance operate within child abduction murder investigations occurring in different locations. Because time and distance are critical solvability factors in child abduction murder investigations, this study provides valuable information for homicide detectives who may need to change investigation strategies and tools to respond to child abduction murder geographic differences.

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Child Abduction Murder, Time to Death, Geographic Differences

E54 An Application of Gunshot Residue (GSR) as Trace Evidence

Jason L. Schroeder, MS, MBA, Harris County Institute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054; William M. Davis, PhD, 1885 Old Spanish Trail, Houston, TX 77573; and Roger Kahn, PhD, Harris County Institute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will understand an application of GSR as a tool in the investigation of gun accidents involving minors. This will occur through the introduction of GSR as an investigative application and a review of accidental cases involving children and firearms.

This presentation will impact the forensic science community by demonstrating the efficacy of GSR as a form of evidence outside homicide and other criminal cases in which it is commonly applied.

Investigators often encounter difficulties with firearm investigations involving minors. These difficulties occur for a variety of reasons and include the emotional nature of the crimes involving children, interference by parents, guardians, and other individuals, as well as legal protections for minors and children. Further, interviewing children associated with the discharge of a firearm is difficult due to the inability of the minor to effectively articulate their involvement or their knowledge of the incident.

The presence of GSR can provide information to an investigation process by associating an individual with the discharge of a firearm. This association may include activities such as firing a weapon and being in close proximity to a firearm during discharge as well as handling a firearm, a fired cartridge, or some other surface bearing GSR. Unfortunately, GSR, like many other types of evidence, cannot conclusively identify a shooter. A negative finding is not exculpatory in nature and a positive test for GSR cannot distinguish a shooter, witness, or victim. GSR testing is most probative in cases in which an individual claims not to have been in the proximity of a firearm during discharge as individuals are not expected to have GSR during everyday activities. While working within these parameters, GSR can provide valuable information for the investigative process.

Ultimately, the analytical conclusions as well as the impact on an investigation must be clearly and accurately represented in a report and subsequently conveyed to all participants in the criminal justice process and possibly to a jury. A thorough understanding of the application of GSR as trace evidence prior to its use benefits all individuals involved in the investigative process. This will be demonstrated via the review of six cases from Harris County, TX. The role of GSR evidence will be put into context with other evidence revealed through independent investigation.

In conclusion, this presentation will provide an overview to the application of GSR through a review of gun accidents involving minors. Additionally, the presentation will provide a review of several cases involving GSR in the investigation of cases involving firearms.

Gunshot Residue, Firearms, Accidents

E55 Forensic Science as an Indispensable Tool in the International Cooperation in Fighting Terrorism

Elazar Zadok, Israel Police, 13 Zadok Street, Holon 5867913, ISRAEL*

After attending this presentation, attendees will appreciate the role of forensic science as a key element in fighting terrorism and producing meaningful intelligence for that purpose.

This presentation will impact the forensic science community by enhancing its awareness of its role in combating terrorism.

Twenty-first-century global terrorism may be described using several important indicators (in bold): (1) **globalization** is the term used to describe changes in the world, especially related to the economy, occurring during the last few decades. Despite its benefits, globalization, unless properly handled, might generate a dangerous anti-security process supporting terrorism development; (2) **modern communication** and the internet allow terrorism to operate without formal organizations and physical bases. These two indicators — globalization and modern communication — may end up in putting power in the hands of small organizations, or even individuals, that not too long ago was reserved only to nations. One important post-9/11 observation is that global terrorism lost all obstacles for **mega-terror activities** and may strike using all available power and means without any constraints; (3) another indicator is that most **terrorism-related intelligence targets** today are individuals, not entities; and, (4) the **war against terrorism is completely asymmetric**. Terrorism is considered successful even if 90% of its activities fail. On the other hand, states fail even if 90% of their anti-terrorism activities succeed.

The years 2000-2004 are remembered as a most intensive terror period in Israel. The Israel National Police dealt with, among other terror events, more than 150 suicide bombers who killed more than 580 people and wounded thousands. This is where the impact of forensic science in fighting terrorism can be fully understood. Three important roles are defined for forensic units in such circumstances: (1) identify as quickly as possible the attacker in order to prevent consecutive terror events; (2) scientifically identify the victims; and, (3) produce valuable intelligence to help fight and prevent additional attacks.

Forensic intelligence is a relatively new concept. It deals with the acquisition and analysis of information extracted from crime scenes, using diversified forensic tools, processing and transforming it into useful intelligence, then using this information to identify criminals and/or prevent additional crimes. During the last decades of international terrorism, terror scenes or events are considered by most relevant agencies as severe crime scenes, and forensic means are gaining more and more importance in their investigation.

This presentation will deal with the two main stages of forensically treating terror events in order to generate valuable intelligence: (1) the forensic investigation of the terror scene itself — how different is it from a "regular" crime scene?; and, (2) the build up of (biometric and other) databases, their usefulness in fighting terrorism, and the importance of international data contribution and sharing in order to maximize their effectiveness in fighting and preventing global terrorism.

Examples taken from terror incidents which occurred in Israel and other countries will be discussed in light of the above information.

Forensic Intelligence, Terrorism, International Cooperation

E56 University Groper: How One Suspect Was Identified Using Touch DNA Findings — A Successful Case Study

Julie L. Valentine, MS, Brigham Young University, 532 SWKT, Provo, UT 84064; and Suzanne Miles, BS*, Utah Bureau of Forensic Services, 4501 S Constitution Boulevard, Taylorsville, UT 84129*

After attending this presentation, attendees will: (1) better understand the benefits of strengthening relationships between forensic nurses, medical evidence collectors, and forensic scientists; (2) realize the importance of DNA evidence collection in stranger-groping assault cases; and, (3) explore changes in evidence collection practice based on more sensitive DNA analysis methods.

This presentation will impact the forensic science community by reinforcing the importance of collaboration between forensic nurses, medical examiners, and forensic scientists in the development of best practice guidelines in sexual assault cases with the advent of more sensitive DNA analysis methods.

This presentation focuses on exploring the benefits of a symbiotic working relationship between forensic nurses/medical evidence collectors with those processing the biological evidence, forensic scientists, to achieve optimal results in DNA findings in sexual assault cases. Additionally, this presentation provides suggestions for evidence collection practice changes based upon the development of more sensitive DNA testing methods and introduces a new evidence collection form developed to capture touch DNA documentation in stranger-groping cases. A case study will be presented on the successful identification of a serial groper from touch DNA analysis findings. An overview of the Short Tandem Repeat (STR) and Y-chromosomal Short Tandem Repeat (Y-STR) DNA results found in this case will be provided as justification for the policy changes and the touch DNA form development.

The successful identification of the suspect in this case study occurred because of a strong collaborative relationship between forensic nurses/medical evidence collectors and forensic scientists. The forensic scientists and forensic nurses in this community meet together frequently to discuss best practice guidelines as is recommended for the benefit of both professions.[1,2] During one of these meetings in 2011, a forensic scientist provided education on more sensitive DNA analysis methods, which would impact the testing of touched evidence. Following this meeting, a forensic nurse collected DNA evidence swabs on a stranger-groping case based upon information gleaned from the forensic scientist. Both STR and Y-STR DNA of the suspect were developed from the collected swabs leading to his guilty plea and incarceration.

A newly developed form, Stranger Touch DNA Documentation, was created following the results from this case as a guide in collecting clothing or other items touched by a stranger during an assault. Hard copies of this state form will be provided to attendees during the presentation. This form directs the evidence collector to list the clothing items touched by the suspect, how and where the touch occurred, and to draw the areas of touch on body diagrams. Groping or assault victims are to carefully remove the clothing avoiding the area of touch. The clothing items are then to be folded with the area of touch placed inward, and the items sealed in a paper bag. Additionally, any external stain body swabs are designated on the sexual assault examination form as possibly containing biological fluids or epithelial cells to provide additional information for forensic scientists, helpful in choosing between STR or Y-STR DNA analysis methods. Multiple presentations to law enforcement agencies and forensic nurses/medical examiners were completed across Utah on the use of this form and the importance of obtaining DNA evidence in stranger-groping or assault cases.

In conclusion, presentation of this case study will encourage collaboration between forensic nurses/medical evidence collectors and forensic scientists, and provide grounds for evidence collection practice changes in sexual assault cases due to more enhanced DNA analysis methods.

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Collaboration, Touch DNA, Documentation

E57 Analysis of Smokeless Powder Components by Ion Mobility Spectrometry (IMS)

Marcela Najarro, MFS, NIST, 100 Bureau Drive, MS 8371, Gaithersburg, MD 20899; and Rose M. Garcia, BS, NIST, 100 Bureau Drive, Gaithersburg, MD 20899*

After attending this presentation, attendees will better understand methods to improve detection algorithms for smokeless powders using IMS.

This presentation will impact the forensic science community by providing a more robust method to detect smokeless powders using IMS.

Smokeless powders are an easily available source of explosives used in the making of Improvised Explosive Devices (IEDs). The bombs used in a recent terrorist attack, the Boston Marathon bombing, used black powder as the weapon of choice. Smokeless powders can be categorized into three classes based on their chemical composition: single-based powder which contains nitrocellulose as its sole explosive propellant ingredient, double-based powder containing nitrocellulose and nitroglycerin, and triple-based powder containing nitrocellulose, nitroglycerin, and nitroguanidine.

There is a need to assess the ability of currently deployed Explosives Trace Detection (ETD) systems to detect a variety of volatile components found in smokeless powders. The threat libraries of current systems already include nitroglycerin, identified by the presence of a NO_2- peak; however, nitro peaks are non-selective as they can derive from many sources making them a common IMS background peak. The goal of this study is to characterize the IMS response of other volatile components found in smokeless powders to improve detection algorithms and provide a more selective identification. Also, single-based powders that do not contain nitroglycerin require the addition of other components to the threat library to illicit an alarm.

Commercial standards of compounds found in smokeless powders were analyzed using Morpho Detection's Itemiser^{Q} Dx to determine compound-specific responses. The instrument was operated in dual mode using default settings and calibrated daily based on the manufacturer's recommendations. Background peaks produced by the Teflon®-coated swabs (matrix) and solvent background were evaluated first in order to subtract these peaks from standard and sample spectra. Standard solutions were diluted in a variety of organic solvents and deposited onto sample swabs to determine whether the instrument response to these compounds was linear. The mass loading analyzed ranged between 0.5ng and 700ng. Commercially available smokeless powders were prepared gravimetrically dissolving the solid powders in a High-Performance Liquid Chromatography (HPLC) -grade solvent. Appropriate dilutions were made to deposit the desired mass onto Teflon®-coated fiberglass swabs.

This study identified seven important compounds found in smokeless powders including Nitro+, DNT+, diphenylamine, methylcentralite, and ethylcentralite by IMS and defined their detection parameters. Some known additives were not IMS amenable, such as potassium nitrate. The measured response curves demonstrate typical IMS behavior, in which there is a linear response at low masses and a saturated or near-saturated response at the higher masses. The reproducibility of the measurements is good as determined by error bars in the response curves (Relative Standard Deviation (RSD) ~10%). Prior to this work, the IMS threat library only contained nitroglycerin and black powder, causing single-based smokeless powders to not alarm. With the addition of these additives to the library, improved detection was achieved. Operating Explosive Trace Detection (ETD) systems in dual mode (explosives/negative and narcotics/positive) is critical since most additives can only be detected in positive mode. Future work includes identifying unknown peaks found in IMS plasmagrams by Gas Chromatography/Mass Spectrometry (GC/MS) and/or Electrospray Ionization/Mass Spectrometry (ESI/MS).

Smokeless Powders, Ion Mobility Spectrometry, Explosives

E58 Examining the Factors Affecting Forensic Scientists' Job Stress and Satisfaction

Thomas J. Holt, PhD, Michigan State University, 655 Auditorium Road, 434 Baker Hall, East Lansing, MI 48824; Kristie Blevins, PhD, Eastern Kentucky University, 521 Lancaster Avenue, Stratton 467, Richmond, KY 40475; Ruth Waddell Smith, PhD, Michigan State University, School of Criminal Justice, 560 Baker Hall, East Lansing, MI 48824; and David R. Foran, PhD, Michigan State University, Forensic Science Program, 560 Baker Hall, East Lansing, MI 48824*

After attending this presentation, attendees will understand the demographic and organizational factors that affect forensic scientists' levels of job stress and satisfaction in a national sample of federal, state, and local crime laboratories.

This presentation will impact the forensic science community by identifying the organizational, managerial, and relational factors that affect scientists' occupational experiences while on the job. These findings can directly inform strategies that may be employed by laboratory managers to improve both working conditions and employee satisfaction.

This study examined a sample of 899 forensic scientists working in public and private laboratories operating at the local, state, and federal level in 25 states across the United States. Data collection took place in two waves, beginning in November 2012 with an electronic survey distributed to all forensic laboratories accredited by the American Society of Crime Laboratory Directors/ Laboratory Accreditation Board (ASCLD/LAB). A second paper survey was distributed in May 2013 to 84 agencies in 25 states to increase the overall response rate and the likelihood of responses from under-represented agencies.

The scientists sampled reported moderate levels of work stress, but moderate to high levels of job satisfaction. Linear regression models were conducted to identify statistically significant relationships between demographic characteristics, organizational characteristics, working environment, managerial and justice system requests, and both work stress and job satisfaction. These models demonstrate that a lack of managerial and supervisory support, poor relationships with prosecutors, increased working hours, and role conflicts led scientists to report higher levels of job stress. Females were also more likely to report higher rates of stress than their male counterparts, and stress levels and factors differed among forensic subdisciplines.

Inverse relationships were identified with respect to job satisfaction. Specifically, scientists who worked fewer hours per week, experienced high levels of support from both supervisors and top management, had low levels of role conflict, and had generally positive feelings about the job were more likely to report greater job satisfaction.

The findings from this study demonstrate that forensic scientists report similar levels of stress and satisfaction to that of other criminal justice system employees, including police and correctional officers, as well as helping professions such as ambulance crews. In order to reduce negative occupational outcomes such as burnout, absenteeism, and job turnover, laboratory managers should implement clear policies to increase flexible scheduling, distribute overtime equitably across scientific staff, establish and maintain clear lines of communication with direct supervisors and management, and have clear guidelines regarding interactions with court staff and prosecutors. There is also a need for greater research on the environmental and occupational experiences of forensic scientists in order to promote their health and well-being in and out of laboratory environments.

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Occupational Experiences, Stress, Crime Laboratory

E59 Neanderthals, Werewolves, and a Pig Man: A Novel and Collaborative Method for Differentiating Human and Animal Skeletal Remains

Brett E. Harding, MBA, District 5 MEO, 809 Pine Street, Leesburg, FL 34748; Barbara C. Wolf, MD, District 5 MEO, 809 Pine Street, Leesburg, FL 34748; Lindsey A. Bayer, MS, 809 Pine Street, Leesburg, FL 34748; and Meryle A. Dotson, MA, District 5 ME Office, 809 Pine Street, Leesburg, FL 34748*

After attending this presentation, attendees will better understand the unique aspects of the forensic evaluation of skeletonized remains, with emphasis on the differentiation of skeletonized human and animal remains. Attendees will also become familiar with a simplified method for differentiating such remains in the course of a multidisciplinary medicolegal death investigation.

This presentation will impact the forensic science community by a providing a simplified, three-part, step-by-step method for distinguishing human and animal skeletonized remains, the principles of which medicolegal death investigators and medical examiners/coroners will be able to apply to skeletonized remains encountered during medicolegal investigations.

Many field investigations are conducted each year to examine and determine the origin of skeletal remains. A large majority of these investigations have been initiated by the discovery of what ultimately are revealed to be remains that are non-human in origin. In a large number of these cases, the bones are scattered and the most distinguishing feature for determining origin, the skull, is absent, fragmented, or obscured. These situations often present a problem for both law enforcement and medicolegal investigators because a reliable method for making such determinations is often not readily accessible. The availability of a forensic anthropologist for immediate response to the scene of the skeletal remains can be unrealistic, if not impossible. Also, the ability of law enforcement to secure a possible crime scene for an extended period of time may be equally problematic.

Medicolegal death investigations vary in type and scope. The goal of obtaining information useful in determining the cause and manner of death can at times be at simple process and at other times a laborious endeavor. These forensic investigations range from the examination of intact bodies to the inspection of fragmented, skeletal, or partial remains. Recognition of the anatomical origin of partial remains can be difficult. Distinguishing animal from human remains in the field can prove to be even more problematic for investigators. Confusion regarding the origins of these remains may result in an inordinate expenditure of investigative time and resources. A survey of the forensic literature reveals few basic field methods for distinguishing faunal from human remains. This presentation introduces a three-part, sequential, medicolegal method for evaluating and determining the origins of partial, decomposed, or traumatized remains in the field. These include scene evaluation, morphology, and osteology. These methods used as part of a multidisciplinary approach will yield more fruitful and effective forensic investigations.

Skeletal Remains, Osteology, Medicolegal Investigations

E60 The Utility of Forensic Evidence in Homicide Cases Tried in London Courts Between 2010 and 2014

Dagmar Heinrich, PhD, University of Huddersfield, Secure Societies Institute, Queensgate, Huddersfield, West Yorkshire HD1 3DH, UNITED KINGDOM; Ruth Morgan, PhD, University College London, UCL JDI Ctr for Forensic Sciences, 35 Tavistock Square, London WC1H 9EZ, UNITED KINGDOM; and Nick Tilley, PhD, UCL Department of Security and Crime Science, 35 Tavistock Square, London WC1H 9EZ, UNITED KINGDOM*

After attending this presentation, attendees will understand the impact forensic evidence had in homicide cases involving sharp implements tried in London courts between 2010 and 2014.

This presentation will impact the forensic science community by demonstrating a method of determining the impact various types of evidence have in court through a mixed-methods approach. This presentation will also highlight how results and conclusions from this type of research can influence stakeholders at all levels of the forensic community: law enforcement, forensic researchers and practitioners, and policy makers.

To date, only limited attempts have been made to evaluate the role forensic science plays in criminal cases and to provide systematic and robust evidence as to its perceived utility. As underlined by the 2009 National Academy of Sciences (NAS) Report, Strengthening Forensic Science in the United States: A Path Forward, in the United States and the House of Commons Science and Technology Committee in the United Kingdom, forensic science research and development is not considered healthy, and calls have been made to develop a new national research budget for forensic science and make it a research priority.[1-3] In light of this, research which seeks to establish the impact forensic evidence has on the criminal justice system and criminal case outcomes is necessary.[2-4] It is key that understanding is developed of the role that forensic evidence plays in the judicial process. Recognizing the role that different types of evidence play in the trials of serious crimes, such as homicides, will potentially allow for evidence-based policy to be formulated as to the allocation of resources.

This study examined the impact of evidence in homicide cases involving sharp implements in London courts between 2010 and 2014. The study distinguished between various types of forensic evidence, such as human biological trace evidence, forensic anthropology and blood pattern analysis, and witness statements and real evidence, and differentiated between prosecution and defense evidence. By evaluating the evidence utilized in these cases and the variables of conviction and associated sentence length, the impact of each type of evidence could be determined.

A mixed-methods approach facilitated the extraction of information from 115 case files and further analysis with regard to verdict and sentence lengths. The study found that certain types of evidence were very prevalent in the homicide cases analyzed, such as testimony, Closed-Circuit Television (CCTV), and medical evidence, while other categories such as forensic chemical and geoforensic evidence were not used. In turn, medical evidence and CCTV were also found to be most significant in moving the mind of the tribunal and conviction of the defendant. Despite hypotheses to the contrary, the most statistically significant findings were in the relationship between types of evidence and sentence length of convicted defendants. Evidential value was established in a relative sense within the cases analyzed in this research, the findings suggesting that medical, CCTV, voice recognition, and defense witnesses had the greatest impact on the adjudication of homicide cases involving sharp implements within the representative sample. Other types of forensic evidence such as shoeprint and biometric evidence were marginally significant in influencing the judgement and sentencing of homicide cases in this research.

Establishing the relative probative value of various types of evidence has contributed to addressing the lack of literature and empirical evidence regarding the impact of forensic science.[2,5] The results of this research allow law enforcement, judiciary, and forensic scientists to identify which types of evidence have the most impact in the adjudication of homicide cases involving sharp implements. This research also provides an empirical foundation for future policy, superseding any current strategies that are grounded in assumptions regarding the utility of forensic evidence. Moreover, this research offers a framework for forensic researchers and policy makers to direct research and resources.

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Forensic Evidence, Homicide, Evidential Value

E61 Identification of Decomposition Odors That Elicit a Response From Trained Cadaver Dogs

Lorna C. Irish, BSc, University of Huddersfield, Queensgate, Huddersfield, West Yorkshire HD1 3DH, UNITED KINGDOM; Gareth Parkes, PhD, University of Huddersfield, Queensgate, Huddersfield HD1 3DH, UNITED KINGDOM; and Anna Williams, PhD, University of Huddersfield, Applied Sciences, Queensgate, Huddersfield, West Yorkshire HD1 3DH, UNITED KINGDOM*

After attending this presentation, attendees will better understand current cadaver dog training practices in the United Kingdom, how trained cadaver dogs react to individual odors associated with decomposition, and how results indicated varying quality and reliability of such dogs.

This presentation will impact the forensic science community by providing results from a controlled experiment in an area with no known previous research. This presentation will add to current research, linking the Volatile Organic Compounds (VOCs) detected from the decomposition process to the responses from trained cadaver dogs.

It is currently not known which chemicals elicit a trained response (indication/alert) from a Victim Recovery (VR) dog; however, numerous studies have identified a wide array of chemicals associated with the decomposition process.[1-3] From these, a number have been identified and characterized by multiple studies using differing methodologies. All these studies broadly confirm that different chemicals are present at specific times/states of decomposition while other chemicals are detected throughout the decomposition process.[4,5]

Results from recent questionnaires initiated by this research and anecdotal evidence from a number of United Kingdom VR dog handlers, indicate that VR dogs will often find human remains in states of decomposition that differ from those with which they have been trained (personal observation). For example, despite VR dogs in the United Kingdom being mostly trained on pork meat in various states of decomposition, they are still capable of successfully locating human remains operationally. This suggests that the chemicals they are indicating on are likely present in both species, or dogs cannot differentiate between pig and human remains and the chemicals they are indicating on are present throughout the decomposition process.

Based on this and a review of the literature, a number of chemicals were identified as being "core" chemicals of decomposition, and possibly those that VR dogs are detecting and that are causing them to give a trained response.[6,7]

It is not currently known if VR dogs indicate on individual, a few, or the whole spectrum of chemicals that give rise to the scent of decomposition.[1] Therefore, to investigate this, an experiment was designed using a number of individual "core" chemicals presented to the dogs in a scent lineup.

Experiments were designed to be comparable to early research that was conducted to determine the chemicals that elicit a response from explosives detection dogs.[8] This was due to ease of set-up and the similarity in the research goals (i.e., determining if individual compounds cause a dog to indicate). It was decided this should be accomplished by presenting a number of individual chemicals detected and identified from the decomposition process in separate vials to a sample of trained VR dogs.

Test chemicals included: ethylbenzene, p-xylene, o-xylene, styrene, nonanal, decanal, carbon disulphide, undecane, dimethyl disulphide, toluene, hexane, trimethylamine, butyric acid, cadaverine, and putrescine. Positive controls (United Kingdom cadaver dog training materials: human bone, pork tissue) and negative controls (chemicals not associated with pig or human decomposition: geranyl acetone, clove oil) were also included in the lineup in addition to blanks.

This research concluded no single chemical achieved the same high response associated with the positive controls within the lineup. Further investigation is required.

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Cadaver Dog, Decomposition, VOC

E62 Houston's Approach: A Final Outcome to the National Problem of Untested Sexual Assault Kits

Irma Rios, MBA, Houston Forensic Science Center, 1200 Travis, 24th Fl, Houston, TX 77002*

After attending this presentation, attendees will get an intimate look at one major city's multidisciplinary, victim-centric approach to testing more than 6,660 rape kits dating back to the early 1980s and the final outcome of processing such a vast number of kits from the perspective of the laboratory, the victims, and different areas of the justice system. Attendees will also become familiar with the project's emerging themes, its challenges, and the ultimate reward of completing what appeared to be an unsurmountable goal.

This presentation will impact the forensic science community by increasing awareness of the benefit of taking a multidisciplinary approach when eliminating a backlog of untested sexual assault kits. It will also improve the response to victims of sexual assault and provide forensic laboratories with information concerning the challenges and workflows required to effectively and efficiently review kits sent for testing at private laboratories.

Backlogged and untested sexual assault kits are a national problem. Many cities have been painted as unresponsive to sexual assault victims. Crime laboratories are under-resourced. Community advocates are stretched thin. Officers have more than their share of calls for service. District attorneys have high caseloads. The impact to these entities when mandated to process all sexual assault kits can be stressful.

The City of Houston, like many others in the news recently, was under the microscope for having a large volume of untested sexual assault kits. For years, Houston's system relied on investigators submitting a request for a rape kit to be tested. Without such a request, the laboratory may not have even known that there was a kit. At times, police didn't request testing because a victim wasn't cooperating, the statute of limitations had expired, consent was a question in the case, or the district attorney refused to issue an indictment.

The National Institute of Justice gave the City of Houston funds to study and address untested sexual assault kits. It was this funding, coupled with city dollars and the passage of a state law in 2011, that led Houston to test all the kits.

The technical reviews and Combined DNA Index System (CODIS) uploads for approximately 6,663 untested kits were completed in February 2015. This was a remarkable achievement. Several themes emerged. Changes have been implemented in response, and the city, along with its police department, prosecutors, and Houston Forensic Science Center (HFSC), have learned that the benefits of testing all kits outweighed the costs. Victim voices will be heard, and long-standing crimes, sometimes crossing state boundaries, will be solved. Sexual assault is a complex crime and requires a cultural shift across the justice system to respond more effectively. This presentation will summarize the strategies that make a multidisciplinary team successful and improve response to rape victims. The strategies presented can be adapted to other municipalities and regions across the country.

Multidisciplinary, Victim-Centric, Untested SAKs

E63 Validation! Validation! Validation!…With a Touch of Reality

Daniele S. Podini, PhD, Department of Forensic Science, 2100 Foxhall Road, NW, Washington, DC 20007*

After attending this presentation, attendees will be informed concerning a project-based learning approach to teach the conceptual and practical aspect of internal validation studies. This presentation will also discuss how "true-mock" cases can have an emotional impact on students and make them understand and appreciate how their jobs as forensic scientists will impact not only society but also single individuals.

This presentation will impact the forensic science community by having better prepared and committed individuals entering the workforce.

As the Combined DNA Index System (CODIS) core loci are about to increase from 13 to 20, forensic DNA laboratories are all tasked with internally validating new Short Tandem Repeat (STR) kits that incorporate the added markers. Validation is extremely time consuming, significantly impacting laboratories' resources. Furthermore, as the sensitivity of STR kits increases together with the increase of mixed-touch DNA samples submitted to forensic laboratories, developing comprehensive validation studies, from which is possible to derive sound interpretation procedures, is becoming more and more important. To address this need, the Forensic Molecular Biology III class at George Washington University (GWU) focuses on validation. Students are tasked with the "internal validation" of a commercially available STR kit. In Fall 2014, the class validated a 5µL amplification of the AmpflSTR® Identifiler® Plus kit, whereas in Fall 2015, the class will develop the internal validation of PowerPlex® 16HS. Starting from the review of the literature and through class discussions guided by the instructor, students design the validation study, then perform it in the laboratory. Each student then independently produces a validation manual describing in detail how he/she determined the analytical threshold, the stochastic threshold, the heterozygote peak balance threshold, mixture ratio, etc.

Understanding validation studies is essential for a DNA analyst to properly interpret data — it is what supports the conclusions that are drawn from the analytical process. Giving the students the opportunity to focus on this important issue and to understand the process and its challenges has proven extremely beneficial for their overall preparation for the job market.

An important component of the Forensic Molecular Biology II class at GWU is the mock case. Students are given properly sealed evidence, which they have to receive, maintaining chain of custody, process, turn it in properly re-sealed, and produce a certificate of analysis/report. Students are also tasked with presenting the case and their findings to the rest of the department (students and faculty) during a graduate seminar session. In the past two years, the students did not know that the evidence mimicked that of a real case. The victim survived and the perpetrators were apprehended and brought to justice, also thanks to DNA analysis. At the end of the presentation, students were told that the facts they described had actually happened and were introduced to the survivor who was present in the audience. The survivor then gave a talk describing how important it was for her recovery to see the perpetrator/s apprehended and then convicted, and how important the job of the DNA analyst (and forensic scientists in general) is to people like her. It has been a very emotional experience for the students.

The reasoning that inspired this approach is that it is often easier to remember an emotion, how something made us feel, rather than a fact. It is a fact that the job of the forensic scientist has an immediate impact on society: closure for a victim, excluding an innocent suspect, or identifying a criminal that would hurt others if not apprehended. The emotional experience of that day will help students always remember that fact

Validation, Inspire, Education

E64 Standard Protocols: Forensic Archaeology Integration With Standard Archaeological and Anthropological Methodologies Following Natural Disasters

Christine L. Halling, MS, Louisiana Department of Justice, 1885 N 3rd Street, Livingston Bldg, 6th Fl, Baton Rouge, LA 70820; Arbie Goings, Goings Consulting Services, LLC, 1503 Vineyard Court, Denham Springs, LA 70726; Ginesse A. Listi, PhD, LSU Geography & Anthropology, 227 Howe-Russell Bldg, Baton Rouge, LA 70803; Ryan M. Seidemann, MA, Louisiana Department of Justice, 1885 N Third Street, Baton Rouge, LA 70802; and Mary H. Manhein, MA, 6511 Jefferson Highway, Baton Rouge, LA 70806*

After attending this presentation, attendees will better understand a non-**Disaster Mortuary Operational Response Team** (DMORT) response scenario that requires a new set of standard operating protocols.

This presentation will impact the forensic science community by demonstrating the need for standard procedures in response to natural disasters when national response teams are not activated.

Forensic archaeology is a discipline formed by the need for practical application of archaeological and anthropological techniques to address unique scenarios involving legal matters. Incorporation of techniques from forensic science coupled with standard archaeological and anthropological methods allows for the creation of new protocols to use after natural disasters ensue. In particular, this presentation suggests how agencies may utilize first responders to create a response plan addressing legal issues regarding human remains and employing recovery teams to ensure that proper documentation, recovery, and identification occurs. Highlighted here are unique problems related to cemetery impacts, and the focus centers on the necessity for both the implementation of legal and scientific efforts early in the process to minimize misidentification, appropriation of remains for trafficking, and the incorporation of bioarchaeologists with experience in archaeological excavation and historical documentation.

When communities are devastated by natural disasters, such as hurricanes, frequently the dead are impacted as well. In South Louisiana, where bodies are often entombed in above-ground vaults, family crypts, or mausoleums, the effects of torrential rain, wind, and flooding that accompany such storms on these cultural features may be acute, sometimes resulting in disinterment. First responders to situations such as this encounter a plethora of legal and logistical problems. In the wake of natural disasters, local and national officials often seek the expertise of forensic archeologists and anthropologists in the recovery of disinterred human remains. Crucial at this juncture is the implementation of protocols that assess the unique aspects of the situation, address the law concerning the dead, and use available expertise from those that specialize in archaeological and anthropological methodologies. While federal response teams such as DMORT have protocols established for handling these concerns, the scale of the disaster's impact and the cost of deployment, even with government subsidies, can be prohibitive for smaller jurisdictions needing assistance. In such instances, the response must be coordinated on a state or local level. This presentation provides an example of one such situation.

Hurricane Isaac hit southern Louisiana in August 2012 as a category 1 hurricane and resulted in some parishes experiencing flooding up to 15 feet. Plaquemines Parish, located southeast of New Orleans along the Louisiana coast, was hit particularly hard. Significant damage to three cemeteries in the area resulted in the displacement of numerous caskets, burial vaults, and crypts and the scattering and commingling of human remains. With no activation of DMORT, a mixture of local, state, and private entities coordinated cemetery response efforts, recovery planning, and assessment of the damage reported in Plaquemines Parish. This coordination was a crucial step in ensuring that proper protocols were implemented and provides a model depicting how multiple agencies working together can solve multifaceted scenarios similar to what is reported here; however, this approach also highlights the need for a local point agent in order to provide full resolution of the recovery effort. This person not only adds an important feature to the recovery team to ensure all operations are concluded, he/she also remains the conduit point for family or agency assistance after recovery and cleanup is completed, and after responders have returned to their normal activities. The use of recovery archaeology can be vital in preserving cemeteries and memorial grounds and, in this case, was necessary for returning skeletal remains to their graves.

A set of uniform response guidelines is proposed that might prove useful to local officials concerning such recoveries. The response to Hurricane Isaac provides one example of a natural disaster that, when handled appropriately, resulted in the documentation of not only human remains, but cultural materials and identification of the dead.

Forensic Archaeology, Hurricane, Disaster Response

E65 A Novel Method for Ninhydrin Development of Fingerprints on Absorbent Surfaces

Howard A. Harris, JD, PhD, University of New Haven, Forensic Science Program, 300 Boston Post Road, West Haven, CT 06516*

The goal of this presentation is to familiarize attendees with a new method of developing fingerprints on absorbent surfaces such as paper. The method is rapid, solvent free, does not run ball point ink, and is easily portable. Fingerprints on paper are particularly useful since they can be recovered weeks or months after being deposited.

This presentation will impact the forensic science community by showing how the contact ninhydrin method makes recovery of fingerprints from paper available to those without laboratory facilities.

This presentation will cover work completed on the development of a novel ninhydrin method for developing fingerprints on absorbent surfaces such as paper. When developing prints without running ink is desired, a dry ninhydrin method has been known for a long time. It was not used regularly since it is such a slow reaction; the recommended development period is 48 hours or more. The primary objectives in this study were to develop a contact ninhydrin fingerprint visualization method free of organic solvents, highly portable, rapid, does not run ink, and which produces high-quality fingerprints on paper and other absorbent surfaces. This is accomplished using three basic components: a ninhydrin development sheet, a "dry" portable moisture source, and the use of microwave energy to speed the usually slow ninhydrin/amino acid reaction. This reaction produces the deeply colored Ruhemann's purple that makes the fingerprint visible.

The first component is treated paper (card stock) containing ninhydrin and additives to enhance contact with the substrate. The second component is the "dry" portable moisture source which can be a diaper pad or a paper package containing a deliquescent solid. The third major component is the use of a sandwich of microwaveable boards to hold the other components together under pressure to facilitate the good contact needed for the solid phase reaction.

This study tested this method with synthetic fingerprint material applied to a substrate with a rubber stamp and fingerprint samples obtained anonymously from students and others. The synthetic fingerprint material, obtained commercially and applied, as reproducibly as possible, to the paper substrate allows one to compare ninhydrin sheets of varying composition and evaluate method variations for their efficiency.

The procedure developed involves placing the substrate, ninhydrin sheet, and moisture source in intimate contact between two rigid materials that readily pass microwaves. The sandwich is held in close contact with heavy rubber bands. This sandwich is placed in a microwave oven set on defrost or low power and microwaved for a short time (two to six minutes). The sandwich is allowed to cool for about five minutes, opened, and the substrate examined. This procedure has been run on a great many samples and has been shown to develop visible prints with samples even when only trace amounts of amino acid containing residue have been deposited.

Some of the advantages of this method are: (1) the proposed "contact" ninhydrin method greatly speeds the color development versus the classic "dry" ninhydrin method; (2) the method does not cause colorization of the substrate background which makes for better contrast; (3) ninhydrin sheets are easily prepared, quite stable, and readily portable; (4) the development process is free of any organic solvents; (5) the ninhydrin sheets can be used multiple times; (6) the proposed method does not cause the running of most ballpoint pen inks; and, (7) all the necessary materials, except the microwave oven, can be transported elegantly in an American Acdemy of Forensic Sciences (AAFS) meeting bag.

The contact ninhydrin procedure should make recovery of fingerprints from absorbent surfaces available to individuals involved in investigation but with limited access to laboratory facilities. Absorbent surfaces have the advantage of providing a source of usable fingerprint evidence months, or even years, after they were deposited.

Fingerprints, Paper, Ninhydrin

E66 Forensic Archaeology and Surface Scatter Body Recovery: A Contested Missing Person Case

Sharon K. Moses, PhD, Northern Arizona University, 555 E Pine Knoll Drive, Bldg 98D, PO Box 15200, Flagstaff, AZ 86011*

After attending this presentation, attendees will have a clearer understanding of complications that can result from non-professionals in charge of and participating in a surface scatter body recovery, as well as issues that arise when county jurisdictions inadvertently prevent information sharing when a protocol is not in place.

This presentation will impact the forensic science community by demonstrating: (1) how simple protocols enable law enforcement to avoid unnecessary delay or failure to link pertinent data in a missing person case across jurisdictional lines; and, (2) the need to exercise discernment and limitations in allowing non-professionals to document a recovery scene.

This presentation will provide an overview of a case that resulted in a lawsuit that changed a state's policy on cross-referencing the data banks of abandoned cars with missing person cases. It is also a cautionary tale intended to motivate establishment of standards, limitations, and parameters for volunteer organizations with little or no professional background in archaeology that assist law enforcement in missing person body recoveries that may later prove to be criminal cases.

It will also demonstrate how to avoid unnecessary delay or failure to link pertinent data relevant to investigating missing person cases across jurisdictional lines. Furthermore, it will show how minimally trained or untrained volunteers participating in a surface body scatter can complicate and negatively affect community and law enforcement relationships and documentation of the scene for future reference.

Forensic archaeologists can offer more to interpretation of a body recovery scene than individuals who have not had the benefit of an in-depth education that equips them with understanding landscape and geological considerations, human behavior, animal behavior, taphonomy, and documenting a body recovery for future reconstruction within a scientific paradigm, should it become necessary.

In Fall 2012, contact was made by a county sheriff's department, its coroner, and by the family of a missing person, for assistance of a forensic archaeologist in a body recovery and professional documentation of the site to quell unrest. A 54-year-old male had been missing for nearly two years until a timber worker stumbled upon a human bone in the course of marking trees for harvest. What followed was a body recovery wherein previously missed opportunities had led to a six-month delay in locating the last known whereabouts of the missing individual, insinuations of a police cover-up, and unfounded speculations by volunteer non-archaeologists involved in the surface scatter body recovery. Furthermore, because of the fomented distrust of law enforcement and due to their ignorance about recovery sites, the volunteers had compromised the recovery area in an effort to make the work of the forensic archaeologist "easier."

Forensic Archaeology, Human Remains, Surface Scatter

E67 The "CSI Effect": The Barristers and the Bench

Janne A. Holmgren, PhD, Mount Royal University, Dept of Criminal Justice Studies, 4825 Mount Royal Gate, SW, Calgary, AB T3E 6K6, CANADA*

After attending this presentation, attendees will better understand the impact of the real and perceived extent the so-called "CSI effect" has, specifically on the barristers and the bench. While research into jurors' understanding of forensic evidence based on forensic-related television shows has been researched extensively, little research has focused on the perception of this phenomenon by lawyers and judges.

This presentation will impact the forensic science community by providing insight into the real and/or perceived "CSI effect" on barristers and the bench. This data provides the background and preliminary findings of how forensic-related television shows might contribute to whether or not the so-called "CSI effect" presides in the minds of not only jurors, but of lawyers and judges and whether or not this perception potentially changes the structure/architecture of a given criminal trial. The presentation outlines possible solutions for judges, experts, the crown, and the defense.

The purpose of this project was to develop insight into the factors that affect and influence lawyers' and judges' perceptions and understanding of the impact of forensic-type television shows. The shows' fictional portrayal of crime scene investigations has prompted real demands for DNA and other scientific evidence from prosecutors and defense lawyers in the courtroom who believe that this type of evidence is warranted. Submissions to forensic laboratories have increased due to the fact that both the prosecution and the defense fear that their cases will be lost to the jury if, for example, forensic evidence is not included as part of the evidence. It is what lawyers and judges refer to as the "CSI effect." This phenomenon was examined relying on the messages from the barristers and the bench. Semi-structured interviews were conducted with 15 lawyers and judges regarding how they are, and have been, affected by the introduction of the "CSI effect" concept.

The findings of this research suggest that potential jurors are educated, but not always correctly, about forensic evidence from watching crime-related television shows; however, the concerns raised in this research are the same for potential jurors, lawyers, and judges. The findings suggest that there is a real expectation for forensic evidence, and that this expectation is shared among all members of the "truth finding committee." Whether there is a real or a perceived "CSI effect" on jurors, this inquiry suggests that there is a "CSI effect" on the bench and the bar.

"CSI Effect", Barristers and Bench, Forensic Science

E68 Implementation of the National Institute of Justice's (NIJ's) Online Firearms Examiner Training Course in Marshall University's Graduate Curriculum and Its Potential to Reduce Time to Competency

Season E. Seferyn, MSFS, Marshall University Forensic Science, 1401 Forensic Science Drive, Huntington, WV 25701; and Pamela J. Staton, PhD, Marshall University Forensic Science MSFS & Center, 1401 Forensic Science Drive, Huntington, WV 25701*

After attending this presentation, attendees will understand the steps taken to implement the NIJ's online Firearms Examiner Training modules into the graduate classroom. Attendees will have an example as to how these modules were implemented into the Marshall University Forensic Science Program and what methods were employed to measure competency.

This presentation will impact the forensic science community by adding another tool to enable future examiners to enter the field with a more comprehensive understanding of the concepts relied upon by the field of firearms and tool marks and how such an approach can aid in the reduction of time until competency.

The presentation will discuss the creation and implementation of a two-semester graduate course which incorporates the NIJ's online Firearms Examiner Training course.[1] Background information will address considerations for adding the course to the graduate forensic science curriculum with regard to overcoming curriculum deficiencies and the program's potential effect on the future competency of the students in the field after graduation. The NIJ's Examiner Training includes a total of 15 modules, including the introduction, history, and safety sections. Subsets of students were created according to whether the student completed one semester, two semesters, or zero semesters of the Firearms course. All students, regardless of their group, completed a 200-question pre-test examination at the beginning of their first semester and a post-test examination at the end of their second semester.

For the students taking the course, quizzes were developed to assess the student's knowledge gained after each module. At the end of the semester, a final exam was taken that covered the material from that semester. It is important to note that the quizzes were not repeats of the questions in the pre-/post-test examination.

Findings revealed that the students who took the American Board of Criminalistics Forensic Science Assessment Test (FSAT) from Marshall University's Forensic Science Program ranked sixth in the country in the Firearms section in 2013 in comparison to the 15 other schools that participated in the examination (prior to the implementation of the course). In 2014, the Marshall University students who completed the FSAT ranked first in the Firearms section out of the 15 other schools that participated in the examination (after the implementation of the course). In addition, positive student growth rates from pre- to post-test examination will be discussed as well as the impact one semester, two semesters, or zero semesters had on the growth. Surveys of graduates working in the firearms field and their supervisors will be presented as well. In summation, attendees will take away the core knowledge needed to implement this modified online course into their university's curriculum.

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Firearms, Online Modules, Competency

E69 Teaching Today's Students: Hybrid Learning

Crystal L. Wagoner, MFS, 3450 Poplar Hill, Clarksville, TN 37043; and Christina A. Leija, MS*, 2419 Orr Drive, San Antonio, TX 78227*

After attending this presentation, attendees will understand the value of hybrid learning and receive tips on how to successfully implement it into their educational programs, including primary, secondary, and continuing education.

This presentation will impact the forensic science community by providing information about an educational delivery method that embraces technology, personalizes education for today's students, maximizes the use of instructional time, and further develops "soft skills" such as time management, critical thinking, and problem solving. This presentation expounds on previous information about "flipped classrooms" by reviewing the advantages (and disadvantages) to hybrid learning, sharing first-hand experiences of implementation, and providing tips for successfully applying hybrid techniques into any type of educational program.[1]

Under the pedagogy of blended learning, the hybrid classroom takes advantage of technology to provide students with a personal educational experience that allows them to access a variety of learning activities at their own pace and convenience while maintaining Face-To-Face (F2F) interaction with an instructor through brick-and-mortar classroom activities designed to reinforce weekly learning objectives. Numerous researchers claim hybrid learning is the future of education because it combines the best of online and traditional classrooms to provide a delivery modality with numerous advantages to students, educational institutions, and employers.[2-12]

As early as 1999, education professionals recognized that access to technology shifted the nucleus of learning away from the traditional classroom; however, educational institutions have been slow to embrace the change, partly due to a "disconnect" between today's educators and learners.[13] Research indicates there are currently three generations of adult learners: Baby Boomers, Generation Xers, and Generation NeXters.[14-17] Generation NeXters have grown up in a technological world and expect it in every aspect of their lives. Generation Xers have been steadily exposed to technology and embrace it. Baby Boomers were born before computers and cell phones were accessible to everyone and a few still tend to avoid them. The majority of educators are Baby Boomers and some are still uncomfortable with technology. In the traditional classroom, the instructor is the "star" because the focus is on lecture. Online, the student is solely in charge of learning and often feels alone or unmotivated.[18-21] In a hybrid learning environment, the instructor's role is that of a facilitator or guide. Learning is now student-focused and requires the use of many different skills to express competency in a field of study. This can be a challenge to technology-impaired instructors and an obstacle to implementation; however, a clear plan with phased implementation can make the move to hybrid learning much smoother and rewarding for everyone involved.[22]

This is a technological world that strives to constantly keep up with the ever-changing world around us. This being said, there is a duty to embrace technology and prepare students for the world in which they will live and work.

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Education, Hybrid Learning, Delivery Modality

E70 Multi-Phase Postmortem Computed Tomography Angiography (MPMCTA): Is an Interventional Radiological Approach Possible Instead of the Classical One? A Preliminary Study

Fatima-Zohra Mokrane, MD, 1 Avenue Professeur Jean Poulhès, 31059 Toulouse Cedex 9, Toulouse 31059, FRANCE; Frederic Savall, Service de médecine légale, Hopital de Rangueil, 1 avenue Professeur Jean Poulhès, Toulouse Cedex 9 31059, FRANCE; Silke Grabherr, PhD, Centre Universitaire Romand de Médecine Légale, Chemin de la Vulliette 4, Lausanne 25 1000, SWITZERLAND; Daniel Rouge, MD, Service de Medecine Legale, Centre Hospitalier Univ Rangueil, Avenue du Professeur Jean Poulhes, Toulouse Cedex 4 31403, FRANCE; Eric Crubezy, PhD, Université Paul Sabatier, 37 Allées Jules Guesdes, Toulouse 31000, FRANCE; Hervé Rousseau, PhD, 1 Avenue Professeur Jean Poulhès, Toulouse 31059, FRANCE; Norbert Telmon, PhD, MD, Service Medico-Judiciare, CHU Rangueil, 1 Avenue Jean Poulhes, Toulouse F-31054, FRANCE; and Fabrice F. Dedouit, 1 Avenue Du Professeur Jean Poulhes, Toulouse Cedex 9, FRANCE*

After attending this presentation, attendees will learn that an interventional approach for MPMCTA is an easier approach for postmortem investigations. This new broad technique in forensic and scientific postmortem investigations will be better accepted if a micro-invasive method is applied. With this study, the feasibility and good quality of such a technique was established.

This presentation will impact the forensic science community by illustrating how an interventional approach is an alternative approach for classic MPMCTA in cases of conventional cannula insertion.

Purpose: MPMCTA is a new diagnostic tool used to diagnose organ and vascular lesions. This technique requires surgical denudation of some anatomical regions followed by insertion of surgical cannulas. The goal of this report was to assess the feasibility of sheath insertion instead of surgical cannula insertion for MPMCTA and to assess the quality of imaging in comparison with the reference standard procedure: the opacification through surgical cannulas.

Materials: The protocol was performed on eight bodies in the hospital's medicolegal. All cases were medicolegal autopsies ordered by the public prosecutor in charge of investigation, except for one scientific autopsy. The control group was composed of eight MPMCTAs produced for a forensic purpose and randomly selected.

Methods: The first step was the sheath insertion for the Interventional Radiology (IR) approach group and surgical cannula insertion for the control group. Then, a conventional MPMCTA was applied, using the three conventional phases (arterial, venous, and dynamic). Vascular opacification quality was studied with a special focus on the dynamic phase. Regions of interest were cerebral veins opacification, main thoracic abdominal and pelvic vessels, and arterial and venous lower limbs opacification. A statistical analysis was applied on these semi-quantitative results, using non-inferiority tests such as the Ficher test.

Results: Feasibility — sheath insertion was possible for each case of the IR group. A global study of the Computed Tomography (CT) examination did not lead to visually significant differences between the IR and control groups. Coronary opacification was optimal in both groups. Abnormal MPMCTA findings on the IR group were easily identified and were confirmed by autopsy. Vascular opacification quality — Multiple steps were performed to assess absence of difference between groups: (1) cerebral vein opacification — this step showed a complete cerebral vein opacification in half of the cases for both groups; (2) global vascular opacification — 16 items were selected from major arteries and veins in the cerebral and cervical, thoracic, abdominal, and pelvic regions. Complete opacification of the 16 items was achieved in six cases from both groups; and, (3) vascular lower limbs opacification — this step allowed studying distal opacification for both groups. Even if the results seemed better with the IR group, they were not statistically significant. This is certainly due to the small number of cases studied (n=4).

Arterial lower limbs opacification: ipsilateral distal opacification was achieved in 25% of the cases for the IR group, while none was achieved using the control group. Contralateral distal opacification was achieved in 25% of the cases for both groups. Venous lower limbs opacification: no distal opacification was seen, using both techniques.

Conclusion: Image quality using the IR approach was as good as that obtained using conventional surgical cannulas. Thanks to this technique change, the approach was micro-invasive rather than mini-invasive. This could be applied especially for scientific autopsies in order to improve the family's acceptance of the autopsy.

Postmortem CT Angiography, Radiology, Interventional

E71 Italian Emergency Medical Team (EMT) Experience Regarding Crime Scene Access: A Proposal for a Specific Training Program

Luciano Garofano, PhD, Accademia Italiana di Scienze Forensi, Via G. D'Annunzio n.9, Parma 43100, ITALY; and Cristina Enrica Brondoni, MS*, Via Volvinio 30, Milano 20141, ITALY*

The goal of this presentation is to inform attendees of the Italian EMT experience regarding crime scene access.

This presentation will impact the forensic science community by comparing the traditional application of crime scene access techniques within the Italian EMT model and could be useful to other EMTs worldwide due to the differences of preparation, experience, and skill of EMTs in other countries.

In Italy, the EMT is different from region to region and every region has its own protocol for approaching patients and emergency situations. Every region delegates non-profit associations to provide emergency service by ambulances. None of these protocols include any particular procedure to enter and operate within a crime scene. In Italy, many of EMTs are volunteers.

Starting from these significant differences among training, experience, and protocols, this study focuses on a specific training program to preserve the crime scene during and after the EMT access. The study was initiated due to statistical data provided by Milan Operation Centre "118" (the Italian emergency number to call for an ambulance) as well as from field operations and newspapers. The conclusion was that none of the protocols followed by the EMTs considered the adoption of a precise method to be applied at crime scenes.

The data highlighted a problem: 90% of crime scenes had been damaged and contaminated by the access of the EMT team. In Italy, the EMT team does not operate if the patient is dead. But in most cases, even if they are dealing with a dead patient on a crime scene, the crime scene is often damaged by EMT access.

The second phase of the study focused on the analysis of EMT crews. The research revealed a heterogeneous collection of protocols, resources, workers, experiences, approaches, abilities, and skills.

In the third phase, a unique method was created to improve or to create the abilities of the EMT crew to enter a crime scene without touching, moving, removing, or destroying forensic evidence. The method consists of two simulated crime scenes for EMT trainees and an introductory explanation of forensic science. At first, the crime scene is presented at the very beginning. Team trainees are invited to enter the crime scene as first responders and to work as they normally would. After the first simulation, a deeper explanation about the forensic aspect and evidence collection is provided.

The trainees teams are then invited to re-enter the same crime scene. In the second entrance, in 95% of the cases, trainees paid attention to possible forensic evidence while they worked, maintaining protocol and preserving evidence.

This method is based on information flow. If EMTs are allowed to recognize and understand forensic work, they can operate taking care not to move objects on the scene or remove/contaminate evidence from the scene while making appropriate photo-video documentation.

Actual criminal cases will be illustrated to show the benefits of the proposed training.

EMT, Crime Scene, Training

E72 Autopsy Rate in Suicide by Poisoning Is Low in Denmark Compared to Finland

Seija Ylijoki-Sørensen, MD, DDS, PhD, National Institute for Health and Welfare, Kytösuontie 11, Helsinki 00300, FINLAND; Jesper L. Boldsen, PhD, ADBOU, Institute of Forensic Medicine, Lucernemarken 20, 5260 Odense S, DENMARK; Lene W. Boel, PhD, Brendstrupgaardsvej 100, Aarhus N 8200, DENMARK; Henrik Bøggild, PhD, Public Health and Epidemiology Group, Niels Jernes Vej 14, 3-209, 9220 Aalborg, DENMARK; Kaisa Lalu, PhD, National Institute for Health and Welfare, Kytösuontie 11, 00300 Helsinki, FINLAND; and Antti Sajantila, MD, PhD, Department of Forensic Medicine, Kytösuontie 11, 00014 Helsinki, FINLAND*

After attending this presentation, attendees will better understand that the limited use of forensic autopsy to confirm the cause of death in deaths classified as suicides indicates that mortality statistics of suicides may not be reliable in Denmark, specifically in cases in which the cause of death is registered as poisoning.

This presentation will impact the forensic science community by illustrating that national differences in the legislation on cause and manner of death investigation are reflected in different national autopsy rates in suicides, although the cause of death is registered as, for example, poisoning.

The consequences for national mortality statistics caused by differences in the legislation on cause- and manner-of-death investigation on deaths classified as suicides in Denmark and Finland are not known in detail. The goal of this study was to analyze autopsy rates in deaths classified as suicides and to identify any differences in investigation practices in deaths in which the cause of death was registered as poisoning.

Data from the Finnish and Danish mortality registries were summarized for the years 2000, 2005, and 2010. Autopsy rates (total, forensic, and medical) and three age groups of the deceased were compared with regard to deaths classified as suicide and with focus on deaths registered as poisoning.

The total autopsy rate for suicides was 99.8% in Finland and 13.2% in Denmark. Almost all were conducted as forensic. In the age group ≥71 years, Danish suicides outnumbered Finnish suicides (410 versus 283). The total autopsy rate was low in this age group in Denmark (5.6%), whereas it was consistently high in Finland (99.6%). Among Danish deaths due to poisonings, the autopsy rate was 89.5% when these were classified as accidental poisoning, but only 20.7% for cases classified as intentional self-poisoning.

This study showed that the limited use of forensic autopsy to confirm the cause of death in deaths classified as suicides raises doubts about the accuracy of the Danish suicide mortality statistics. This finding is emphasized by those cases in which the cause of death was registered as intentional self-poisoning. The reasons for the alarmingly low interest in performing autopsies on elderly people who commit suicide remain unclear. A more plausible explanation is that the deceased person suffered from (serious) illness, which, in general, is more common for the older population, and therefore, suicide could be considered more acceptable. Overall, the high number of suicides among the elderly in Denmark is striking. Further investigation is needed to show whether the reported number is accurate or whether some deaths were incorrectly classified as suicides. It must be assumed that the Finnish mortality statistics for suicides are more reliable due to the high autopsy rate.

Autopsy Rate, Suicide, Forensic Autopsy

E73 A Survey of Abuse of Illicit Drugs in Punjab, Pakistan

Sardar Ali Wattoo, MPhil, Punjab Forensic Science Agency, Old Multan Road Thokar Niaz Baig, Lahore, Punjab 53700, PAKISTAN; Muhammad Taimoor Chaudhary, MPhil, Punjab Forensic Science Agency, Thokar Niaz Baig Multan Road, Lahore, Punjab, PAKISTAN; and Mohammad A. Tahir, PhD, Punjab Forensic Science Agency, Thokar Niaz Beig, Multan Road, Lahore, PAKISTAN*

After attending this presentation, attendees will be aware of recent tendencies in drugs of abuse and the local names of certain drugs of abuse available in Punjab, Pakistan. This presentation will highlight the recent trends of drug abuse in Pakistani society ranging from lower class to upper elite class.

This presentation will impact the forensic science community by providing a detailed picture of narcotic and controlled substance abuse with relevance to the socio-economic condition of people in various areas of Punjab, Pakistan. Understanding these trends of drug abuse in Pakistan is important as Pakistan is a major trafficking route for narcotics.

The use of drugs in a manner or amount inconsistent with the medical or social patterns of a culture is called drug abuse.[1] The abuse of narcotic drugs is one of the major socio-legal problems of Pakistan. The number of drug abusers and deaths resulting from drug abuse is increasing daily in Pakistan. At the time of independence in 1947, the approximate number of opium users was 100,000, mostly belonging to lower socio-economic groups because of the low cost of opium.[2] "The Golden Crescent" (Afghanistan, Iran, Pakistan), "the Golden Triangle" (Laos, Thailand, and Myanmar), India, Lebanon, and Mexico are most commonly involved in the illicit opium trade.[3,4] In Pakistan, agencies involved in the seizure of controlled substances include the police, the Anti Narcotics Force (ANF), the Frontier Corps (FC), Pakistan customs, Pakistan Rangers, the Airport Security Force (ASF), and the Pakistan Coast Guard.[5] The Narcotics Unit of the Punjab Forensic Science Agency is the only functional forensic drug chemistry laboratory in Punjab province and receives thousands of cases per year.

A retrospective data survey was conducted at the Punjab Forensic Science Agency to evaluate the current drug abuse patterns in various districts of Punjab. For this purpose, data on narcotic cases received at the laboratory from May 2012 to May 2015 was evaluated. The data was categorized on the basis of the type of narcotic samples received and the number of cases received from each district of Punjab.

Among all cases (44,237), the majority of the cases were cannabis resin (30,131), followed by heroin (12,195), opium (1,551), and various pharmaceuticals and other controlled substances (360). Hash resin, heroin, and opium are the most commonly abused drugs in Punjab. A national survey regarding drug abuse in Pakistan revealed that approximately 50% of addicts use heroin, 28% charas, 6% opium, 5% alcohol, 2% bhang, 1% tranquilizers, and so forth. Poly-drug abuse to maintain dependence is another alarming point in Pakistani society. Drugs most commonly abused in combination include opiates, barbiturates, tranquilizers, and other legal psychoactive drugs.[2] The abuse of narcotic drugs was more prominent in the districts of Lahore, Faisalabad, Gujranwala, Jhang, and Sialkot. The abuse of cannabis resin was much higher in remote and suburban areas due to its low cost and easy availability. Liquid marijuana (locally called bhang) was mostly abused by people living in the peripheries of grave yards. Submitted heroin samples contained the bulk of cutting agents, namely chlorpheniramine, diazepam, phenobarbital, acetaminophens, etc. The number of cases of club drugs received at the laboratory is relatively low due to their high cost, intricate accessibility and the absence of clandestine laboratories in Punjab. The abuse of club drugs like MDMA, cocaine, and methamphetamine was more prevalent in upper-class areas of Pakistan, such as Islamabad.

The most frequently abused drugs in Punjab include cannabis resin (locally called charas), heroin, opium, crushed poppy plant (locally called bhukki), cocaine, methamphetamine, MDMA, and various pharmaceutical dosage forms of controlled substances (tablets, capsules, injections, syrups, suspensions, etc.), respectively. The extent of the abuse of illicit drugs was related to the socio-economic conditions of the people of Pakistan.[2] Keeping this presentation in mind, preventive measures should be adopted to control trafficking of illicit substances both into Pakistan and through Pakistan to the rest of world.

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Narcotics, Club Drugs, Golden Crescent

E74 Brazilian Federal Police (BFP) Forensic Activities in the Paleontological Area

Guilherme H.B. de Miranda, Diretoria Técnico-Científica/Polícia Federal, Instituto Nacional de Criminalística, SAIS Q. 7 - Lote 23, Brasilia, Distrito Federal 70610-200, BRAZIL; and Camilla Vasconcelos Kafino, MS, Brazilian Federal Police, Instituto Nacional de Criminalistica, SAIS Q. 7 Lt 23, Brasilia 70610-200, BRAZIL*

After attending this presentation, attendees will understand some of the aspects of the BFP's forensic actions related to fossils. The main objective of this research was to consolidate existing information on the fossils seized by the BFP, which were the subject of the BFP forensic scientists' examinations between 2005 and 2014.

This presentation will impact the forensic science community by describing and analyzing the recent paleontological forensic activities of the BFP. Although only a few dozen are discovered in a decade, the forensic examination of fossils is very important and requires special attention because of the high scientific and cultural value.

In the context of the BFP, all the technical documents produced by the forensic scientists are recorded and stored in a national forensic database system (Sistema Integrado de Investigação Criminal (SISCRIM)), which contains more than 20 million records and more than 720 thousand documents. The Federal Forensic Science Body of the BFP is composed of a central unit (the National Institute of Criminalistics), in Brasilia, and 51 decentralized units, present in all 27 Brazilian state capitals and 24 other strategic cities throughout Brazil.

Brazilian fossils are considered by law as public goods and described as Union heritages. They are legally protected on a federal scale and their trade is prohibited. One of the newest lines of investigation of the BFP is the fight against environmental crimes. For instruction of police and judicial investigations, forensic scientists, with the assistance of paleontologists, perform tests for the identification and description of specimens and fossils of animals and plants, which have been seized due to illegal trade or possession.

A survey was conducted of forensic reports on fossils seized in Brazil. In all, 141 documents were identified (137 reports and 4 technical information items), concentrated in 13 forensic units. A single local unit (Universidad de Ingeniería y Tecnología (UTEC) of Juazeiro do Norte/Ceará), in the Chapada do Araripe, Northeast region, was responsible for 43 of the documents (30.5%). The second, in quantitative terms, with 31 documents (22%) was the forensic unit of São Paulo State. The National Institute of Criminalistics was third with 22 documents (15.6%).

Currently, there are nearly 1,100 forensic scientists working in the BFP on several modalities of forensic science. These are professionals with varied academic backgrounds (18 distinct areas), highly qualified (about one-third of them are postgraduate, at least 80 doctors), well screened (by public contest), and professionally motivated (the BFP is one of the public institutions with greater respect and prestige in Brazilian society); however, there are no paleontologists on the BFP staff and fossil surveys are usually performed by trained experts in related fields, such as geology and biology. The documents examined were produced by 36 forensic scientists (16 of which acted as first author). In cases of greater complexity, the forensic team generally requests assistance and guidance from paleontologists in universities or in the federal agency responsible for the regulation and supervision of mineral matters, the National Department of Mineral Production (DNPM).

The reports examined were classified into 15 categories, according to subject: pseudofossils (6); fish (41); plants (46); mollusks (26); arthropods (14); trilobites (7); bryozoans (1); echinoderms (3); *Mesosaurus* (2); mammals (4); birds (2); dinosaurs (3); paleontological sites (45); reptiles (4) ; and amphibians (2). Several reports addressed more than one group of fossils. The number of pieces analyzed per report varied from one to hundreds.

Most of the fossils examined consisted of fish, arthropods, and fossilized trunks. The most common geological source of the animal fossils examined are the Cretaceous rocks of the Santana Formation of Chapada do Araripe, with an estimated age between 100 and 150 million years. For the fossilized trunks, there are two known geological sources: the Santa Maria Formation, Upper Triassic (225 million years old) and the Permian Pedra de Fogo Formation (290 million years old).

Considering the legal character of fossils as national heritages, a common practice adopted by the forensic team at the end of the examinations involving fossilized items is that the objects are recommended to be forwarded to teaching and research institutions, museums, and public collections; however, it should be noted that this is a decision that rests with the judge.

Fossils, Brazilian Federal Police, Paleontology

E75 Time-Dependent Changes in Human and Chicken Bones in Soil Examined by Infrared (IR), Raman, Inductively Coupled Plasma/Optical Emission Spectroscopy (ICP/OES), and Organic Elemental Analysis

Matthew J. Danker, BS, 2450 Lake Road, #907, Huntsville, TX 77340; Donovan C. Haines, PhD, Sam Houston State University, Box 2117, Huntsville, TX 77341; and Joan A. Bytheway, PhD, Sam Houston State University, College of Criminal Justice, Box 2296, Huntsville, TX 77341-2296*

After attending this presentation, attendees will better understand how changes in the organic composition of bone buried with associated muscle and tissues decays as measured by several important analytical techniques.

This presentation will impact the forensic science community by providing an extended data set that builds upon previous literature showing linear time-dependent changes in the organic content of bone as measured by Raman spectroscopy.[1] For applicability in a forensic context, this study examined bones exposed to an outdoor environment in contrast to the previous study that was conducted in an indoor, controlled environment.

Human bone consists of approximately 70% inorganic material and 30% organic material. The inorganic material is mostly the composite hydroxyapatite, which is comprised of calcium and phosphate. This links to the organic material, collagen, and the combined composite gives the bone its strength. Collagen is the predominant organic material within bone but other organic materials, including support proteins and lipids, have been established. In forensic science, the process by which bone decomposes within a soil environment is known as diagenesis. Diagenesis effectively alters the proportions of organic and inorganic components within bone by the exchange of chemical components from the soil to the bone or vice versa. A study found that the relationship between the organic matrix and inorganic matrix of bone samples have shown a linear change over the space of three months by Raman spectroscopy.[1] In another study, inorganic concentrations of calcium, phosphate, potassium, and several others were measured in various soil samples where human decomposition had occurred.[2]

In this experiment, bone samples from chickens and humans, with associated tissue and muscle, were sectioned with a saw and scalpel. The bone and associated soft tissue samples were then buried in an outdoor piney wood environment at the Southeast Texas Applied Forensic Science (STAFS) Facility, Sam Houston State University, Huntsville, TX, at a depth of six inches. The samples were harvested at 2-week intervals for 12 weeks. The bone samples were then studied by IR and Raman spectroscopy to determine the ratios of organic to inorganic material. Soil samples were taken at the same time intervals. The soil samples were taken at three sites for each bone sample: the surface soil above the buried bone, soil immediately adjacent to the bone, and an area in the same environment where no known human decomposition had occurred. The soil samples were analyzed by elemental analyzer for the amount of carbon and nitrogen present in the soil to test the amount of organic material and by ICP/OES to measure the inorganic components in the soil. The ICP/OES and IR instrumentation and support were provided by the Texas Research Institute of Environmental Studies (TRIES), Sam Houston State University, Huntsville, TX.

ICP/OES and the element analyzer preliminary analyses of soil with pre-existing human burial sites at the STAFS facility found there was a significant difference between soil in which decomposition had occurred and virgin soil. Time-dependent results for ongoing experiments measuring the leaching of bone components into soil will be presented.

In conclusion, this study provides preliminary analysis of time dependency of human and turkey bone decomposition via IR, Raman, ICP/OES, and organic element analysis. Future studies will deconvolute the organic components involved using additional analytical techniques including Gas Chromatography/Mass Spectrometry (GC/MS) of bone lipids that may migrate into the soil.

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Decomposition, Diagenesis, Analysis

E76 Human Bias in the Case of JonBenet Ramsey

Claudia M. Bonilla, 8440 Easton Commons Drive, Apt 615, Houston, TX 77095; and Ashraf Mozayani, PharmD, PhD, Texas Southern University, 3100 Cleburne Avenue, Houston, TX 77004*

After attending this presentation, attendees will understand characteristics of human bias, factors of contextual influences, how human bias can affect the field of forensic science, and how the public's perception can be distorted due to contextual influences.

This presentation will impact the forensic science community by serving as an aspect in understanding the importance of human judgment and how it can greatly persuade the process of forensic analyzation and the public's perception of forensic science and justice. It is highly recommended that all forensic analysts, police officers, and laboratory specialists become very familiar with the potential hazards of human bias.

This study will present a case study that has scrutinized the use of forensic science with examples of errors in human judgment, evidence collection, processing and interpretation, the dangers of outside influences, and society's views on the consequences as it pertains to solving criminal cases and justice. This presentation will also share potential solutions in order to correct or minimize negative feedback as it pertains to the field of forensic science. These solutions include advancements in crime scene investigation such as appropriate training in collection techniques, written procedures, acceptable standards, and well-educated personnel.

There are many cases all over the world that have caused heartache due to botched investigations, bias association, and inadequate skills and/or judgment in crime scene processing, collecting, and analyzing. This research examines the new techniques and standards that have undoubtedly increased the significance of crime scene investigation and forensic analyzation.

In cases such as the 1996 murder of JonBenet Ramsey, errors in investigation methods, evidence analysis, and human judgment have been shown to leave long-term effects in not only the field of forensic science but also the criminal justice system as a whole. The use of excessive media coverage, wealth, and misleading information are just a few factors that constantly contribute to the alteration of public perception The term "contextual influences" can most often be noted as the outside sources that can contribute to the alteration of human thoughts, ideas, and/or judgments. Misleading and leaked information has been shown to sway society more than the world wants to notice. Once falsified data is released, the process of separating the incorrect information from the correct data is almost impossible. Altered perceptions from a visual perspective are shown when there is too much media coverage. Excessive media coverage affects the public by allowing people to interpret favoritism or bias thoughts when television broadcasts show potential suspects under a "different" light. Wealth, on the other hand, can "buy" justice and innocence even if a person can bluntly be seen as guilty.

In many cases, both criminal and civil, the content of contextual influence varies greatly. These types of influences can contribute to the process of decision-making, which more often than not involves substantial consideration and various factors that can affect the final conclusion. Because judgment relies on brain function and interpretation, perception and judgment are a critical part of the human brain that cannot be replaced. But, by gaining knowledge and experience, the brain is more than capable of correcting issues and making careful decisions. By providing effective training in human bias and analyzing and understanding errors in previous cases, forensic analyzers and investigators have shown the capability to interpret crime scenes more clearly and effectively.

It has been nearly 20 years since the death of JonBenet Ramsey and the effects of her case, as well as many others, have permanently left their mark in the field of forensic science; however, over the years, forensic scientists and forensic investigators have learned to improve and develop their skills in order to secure a positive role not only in the eyes of society but also in the justice system.

Forensic Science, Human Bias, Public Perception

E77 Discrimination of Ginseng Cultivation Regions With Stable Isotope Ratio and Multi-Element Analyses

Jisook Min, 10, Ipchun-ro, Wonju-si, Gangwon-do 26460, SOUTH KOREA; Dae-jun Ahn, PhD, National Forensic Service, 10, Ipchun-ro, Wonju-si, SOUTH KOREA; Hye-jin Choi, PhD, National Forensic Service, 10, Ipchun-ro, Wonju-si, SOUTH KOREA; Joo-Hyun Song, MS, National Forensic Service, 10, Ipchun-ro, Wonju-si, SOUTH KOREA; Jae-Hoon Yu, MS, National Forensic Service, 10, Ipchun-ro, Wonju-si, SOUTH KOREA; Jungseok Seo, PhD, National Forensic Service, 10, Ipchun-ro, Wonju-si, SOUTH KOREA; and Dae-Hong Hong, BS, National Forensic Service, 10, Ipchun-ro, Wonju-si, SOUTH KOREA*

After attending this presentation, attendees will better understand pretreatment methods for analyzing soil and ginseng, as well as the correlation of the stable isotope ratio between ginseng and soil. In addition, attendees will learn about the influence of the elemental composition of the soil on the composition of ginseng.

This presentation will impact the forensic science community by explaining how the importance of hydrogen and nitrogen isotope ratios can be used to discriminate the regional origin and elements in soil, and how Discriminant Function Analysis (DFA) is a good tool for forensic analysis.

Korean ginseng is considered to be a precious health food in Asia. Because of this usefulness, criminal acts, including the origin or stealing of ginsengs are rampant. Therefore, this study to investigate the regional origin of ginsengs was required. In this study, two different types of samples were prepared: ginseng and soil from the periphery of ginseng farms. Ginseng and soil samples were collected by the following method. Several regions were selected on the basis of Korean regional criteria (city, county, and district), and two ginseng farms were randomly selected from each of the regions. Next, four to six samples of ginseng and soil were acquired from each ginseng farm. Measurements were then performed in two different ways: (1) the stable isotopic composition of hydrogen, oxygen, carbon, and nitrogen were obtained using Elemental Analyzer/Isotope Ratio Mass Spectrometer (EA/IRMS); and, (2) multi-element analysis was conducted with an X-Ray Fluorescence (XRF) spectrometer. According to the isotope ratio analysis results, hydrogen, oxygen, carbon, and nitrogen isotope ratio values were between -69.16‰ and -30.59‰, 28.11‰ and 41.38‰, -28.96‰ and -22.67‰, and -2.33‰ and 11.81‰, respectively. The hydrogen isotope ratios could be used to distinguish large regional differences (e.g., inland or coastal areas might be divided) by an independent sample *T*-test and the nitrogen isotope ratios showed characteristic information regarding the farms from which the samples were obtained, again using an independent sample *T*-test. In addition, multi-element analysis, based on Discriminant Function Analysis (DFA), showed a successful classification is possible for each region. Furthermore, correlations of stable isotope ratio (carbon and nitrogen) and element quantity are found between ginseng and soil data. Thus, stable isotope ratio values and multi-element analysis could be used to differentiate samples according to regional differences. Therefore, stable isotope (hydrogen, nitrogen) ratios and multi-element analysis may be a useful tool to discriminate the regional origin of Korean ginseng.

Ginseng, Stable Isotope Ratio, Multi-Element Analysis

E78 Association Between Volatile Organic Compounds and Microbes Present During the Decomposition of a Cadaver

Todd A. Deyne, BsC, 1514 Avenue N, Huntsville, TX 77340; Donovan C. Haines, PhD, Sam Houston State University, Box 2117, Huntsville, TX 77341; Aaron M. Lynne, PhD, Box 2116, LDB #300, 1900 Avenue I, Huntsville, TX 77341; and Sibyl R. Bucheli, PhD, Sam Houston State University, Dept of Biological Sciences, Box 2116, Huntsville, TX 77340*

After attending this presentation, attendees will better understand how Volatile Organic Compounds (VOCs) and microbe identification could be utilized in establishing the time of death of a victim and learn about correlations between these two datasets.

This presentation will impact the forensic science community by possibly providing a new means of identifying the time of death in an underutilized way. This presentation will add to the research that is being carried out in forensics, chemistry, and biology by confirming and expanding knowledge of the VOCs that are established in the literature as being released during decomposition. These compounds, along with the microbes present during decomposition, can be used as a means of establishing time of death, but the two datasets are not completely independent and important correlations between the two will be discussed.

The ability to identify VOCs from cadavers during decomposition can lead to newer advancements in forensic science. During decomposition, larger biological macromolecules are broken down into their basic components and some VOCs are intermediates of decomposition. Once the processes of the living body stop, enzymes will go unchecked, causing cells to be lysed from the inside out, a process known as autolysis. This destruction of the cells provides nutrients for the growth of microbes that are already present or are ushered in from the external environment.

VOC samples were taken from decomposing store-bought chickens and from human cadavers via Solid-Phase Microextraction (SPME); two cotton swabs were used to collect microbes. The cotton swabs were contained in two separate tubes, one for the mouth area and one for the belly region of the cadavers. The analysis of the samples was conducted by gas chromatography/mass spectrometry for the VOCs and the cotton swabs were sent to Baylor College of Medicine for sequencing via Polymerase Chain Reaction (PCR). The mass spectra of VOCs were identified and confirmed utilizing the National Institute of Standards and Technology 2008 (NIST08) database, Automated Mass Spectral Deconvolution Identification System (AMDIS), and quantified with the untargeted metabolomics tool, Metabolomics Ion-based Data Extraction Algorithm (Met-IDEA). Data for identified compounds were cross-referenced with ChemSpider or the NIST database for structures and boiling points. All statistical data discussed was calculated using R-console and Statistical Package for the Social Sciences (SPSS) to find the probability statistics between the two (VOCs and microbes). Using the relative populations of specific microbes present and time lapse as a basis, a comparative analysis was performed to identify a possible link between VOCs detected and microbes present during the decomposition process.

Microextraction, Microbe, Volatile

E79 The Use of Lean Principles in a Forensic Environment to Facilitate Transformation

Zo-dee Ledger, 6 Wootton Green Lane, Balsall Common, West Midlands, UNITED KINGDOM*

After attending this presentation, attendees will understand how a continuous improvement technique, such as Lean, and the use of a toolkit can be used to change their processes to improve the service/product they offer to the customer and the efficiency gains they could look to achieve.

This presentation will impact the forensic science community by reducing the turnaround time (TRT) of a case, improving customer satisfaction, and reducing the cost per case/examination. Application of a continuous improvement framework can lead to more efficient outcomes for the criminal justice system.

The Biology Forensic team at the LGC Tamworth laboratory report cases for a range of English and Welsh police forces with differing contract requirements. Two of the contracts have particularly challenging TRTs for a completion of a case, typically between 4 and 14 days, depending on the type of work requested and the type of results obtained as the case progresses. TRT success prior to the Continuous Improvement project was variable and in some instances resulted in service credits (10% of the cost of a case) being paid to the customer for not meeting the required TRT. A project was set up to find a solution for dealing with these challenging TRTs in order to meet the requirements of the contract and deliver good customer service.

A team of four individuals were given one week to review the current process for reporting biology cases and design a new process to meet the challenging TRTs. The team was trained in Lean principles and the use of a toolkit and this learning was used to facilitate the process change. Data was analyzed, the current process was mapped in a flowchart, "waste" was identified in the process, and a root cause analysis was also undertaken. Once knowledge of the current process had been ascertained, a new process was designed which removed as much "waste" as possible. The proposed new process was tested and data was collected to determine whether the changes made an impact on the TRT success.

The changes made to the process did not require any financial investment or any increase in resources. The changes were largely a different way of approaching the work to reduce the waste that was present in the process. The amount of waiting time (no activity on a case) in the process was reduced and the number of handovers (movement of the case file from one individual to another) was also reduced, leading to a more efficient process. As a result of the process changes, TRT success rates for the two most challenging customer contracts improved from approximately 75% to >95%, and therefore improved customer satisfaction. The results show what a group of individuals with good knowledge of a process, the right tools, and a little time can achieve. The approach taken is part of a longer-term culture of continuous improvement and the use of Lean to improve everything that is done for the customer, to motivate staff to improve a work environment, and to make efficiency gains.

Continuous Improvement, Lean, Change

E80 Motor Vehicle Crash or Auto-Pedestrian: Are Stranded Motorists (SM) Left "Stranded"?

Stacy A. Drake, PhD, MPH, The University of Texas Health Science Center, 6901 Bertner Avenue, #748, Houston, TX 77030; and Dwayne A. Wolf, MD, PhD, Harris County ME, JAJ Forensic Center, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will differentiate between motor vehicle crashes, auto-pedestrian fatalities, and SM deaths. Attendees will understand the need to differentiate the SM death from other categories of vehicular fatalities and will identify one method for documenting SM deaths for purposes of data abstraction.

This presentation will impact the forensic science community by serving as an example of how medicolegal death investigation agencies can aid in better categorizing trauma deaths for purposes of stratification of injury data, with the long-range goal of injury prevention in their communities.

Every day thousands of people travel the freeways and hundreds of these become stranded. Regular highway travelers will encounter a stranded motorist or become a stranded motorist. Non-intentional injuries are a leading cause of death. Categories of non-intentional injuries tracked by the Centers for Disease Control and Prevention (CDC) include motor vehicle crashes, auto-pedestrian fatalities, and water incidents.[1] Current literature identifies risk factors and prevention strategies for motor vehicle crashes and vulnerable road users including pedestrians; however, scant literature provides risk factors or prevention strategies for the SM or those coming upon the SM.[2-6]

To fill this gap, a pilot case series was conducted.[7] SMs are defined as any occupant of a vehicle that is stopped in or on the side of a public road. Forty-six SM deaths in Harris County, TX, were identified between 2004 and 2014. Of those deaths, 74% occurred while outside the vehicle. The majority of motorists became stranded due to mechanical problems with the vehicle (67%). Hispanics represented the majority of SM deaths (41%), followed by Caucasians (28%).

Although the SM pilot study identified interesting trends and characteristics, it was limited by the small sample size; however, anecdotally the number of SM deaths in Harris County is higher than these numbers reflect. In other words, the small sample size resulted from an inability to retrospectively identify SM, rather than an actual paucity of SM fatalities. This was because these cases are routinely characterized as "pedestrian" fatalities, or "vehicle occupant" fatalities in medical examiner practice and are not identified by any field on the standard death certificate.

Because accurate identification of the SM subpopulation has implications for public health measures that may ultimately impact public safety (e.g., "move over laws" or even changes in road or vehicle design), measures to prospectively identify and track incidents of SMs were initiated. These measures included the addition of a drop-down box within the electronic investigators' software (PathAssist) that allows prospective identification and retrospective data gathering; this was supplemented with training for the investigators regarding the definition of SM, the importance of identification of the SM, and the appropriate use of the drop-down box. The result of these changes was the identification of a much larger population of SMs. In the ensuing year following implementation of this tracking method, preliminary data indicates that 15 SMs were identified between May 2014 and May 2015. Compared to only 46 SM deaths identified in the preceding ten years, this suggests that a simple tracking measure allows a much more comprehensive identification of this population of vehicle-related deaths. A similar categorization of SM non-fatal incidents was undertaken within local trauma hospitals. Preliminary data indicates that similar to fatalities, a much larger population of these incidents exist than could previously be identified. Studies are now ongoing to compare demographic characteristics and injury patterns of fatal vs. non-fatal incidents of SM crash occurrences.

The implementation of a simple tracking measure to identify the population of SMs is worthwhile, cost-effective, and can be initiated within any medicolegal death investigation system. Attendees will understand the importance of accurate identification of the SM population, and will hear of implications for public health and community safety. Ultimately, a more in-depth understanding of the SM population rests on accurate identification and characterization.

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Forensic Science, Motor Vehicle Crash, Auto-Pedestrian

E81 Forensic Podiatry and Human Identification — The State of This Art in European Countries

Pablo Martinez-Escauriaza, Av Sabino Arana 45, Bilbao, Basque Country, SPAIN; Sara C. Zapico, PhD, Smithsonian Institution, Dept of Anthropology, NMNH, MRC 112, 10th & Constitution Avenue, NW, Washington, DC 20560; and Joe Adserias, DDS, PhD, C/ Balmes 62, Barcelona, SPAIN*

After attending this presentation, attendees will better understand the present situation of forensic podiatry in European countries, in order to initiate its use in forensic casework.

The presentation will impact the forensic science community by illustrating the usefulness of forensic podiatry as a tool for human identification.

Forensic podiatry is defined as the application of podiatry knowledge and experience in forensic investigations: (1) to show the association of an individual with a crime scene; or, (2) to answer any other legal question related to the foot or footwear that requires knowledge of the functioning foot anatomy and biomechanics. Although the scientific aspects of podiatry knowledge are used in clinical practice, the application of this knowledge to the forensic practice must be a cautiously approached practice.

Forensic podiatrists assist in the identification of perpetrators of crime in which bare footprints, footwear, and Closed Circuit Television (CCTV) evidence are involved. The tools used to assess the individual's identification consists of: (1) the effects of foot and lower limb function; (2) the evaluation and matching of wear associated with the foot/shoe interface; and, (3) comparisons based on shoe size.

In Europe, forensic podiatry is an optional subject in Bachelor of Science (BS) Podiatry programs at the universities. Basically, training in this field is carried out through postgraduate programs like the Master of Science (MSc) in Forensic Podiatry at the University of Huddersfield and Salford University. Scientific societies offer certificates of different competencies; for example, the Chartered Society of Forensic Sciences offers the Certificate of Professional Competence – Forensic Podiatry Bare Footprints. In addition, forensic podiatry is represented in different forensic entities such as the International Association for Identification, Fingerprint International Scientific Corporation, and the International Criminal Police Organization (INTERPOL).

Pedal evidence can comprise a number of different forms related to the static or dynamic foot as well as the type of footwear. Footprints, shoeprints, shoes, and their track and trail are evidences that can be easily found in crime scenes. Their collection must be accurate to be able to study the individual's pedal characteristics, leading to the individual's identification. Moreover, biomechanics can also be used for identification through individual walking traits. Currently, neonatal units in hospitals are responsible for taking the imprint of the footprint of newborns. The imprint goes into the annals of hospital documentation and eventually deteriorates. To avoid this, one of the current suggestions is to have a podiatrist or doctor at the time of delivery take those footprints and include them in a digital record.

Even though forensic podiatry can be of great help in crime scene investigation, its representation in European police departments is not that extended.

In conclusion, this presentation highlights the contributions of forensic podiatry toward individual identification.

Forensic Podiatry, Footprints, Biomechanics

E82 Craniofacial Analysis of 3D Computed Tomography (CT) Models and a New Method for Dense Facial Tissue Depth Mapping: A Collaboration Between Forensic Science Researchers and Forensic Art Practitioners

Terrie Simmons-Ehrhardt, MA, Virginia Commonwealth University, 312 N Shafer Street, Richmond, VA 23284; Catyana R. Skory Falsetti, MFS, Maricopa County Attorney's Office, 301 W Jefferson Street, Phoenix, AZ 85003; and Christopher J. Ehrhardt, PhD, Virginia Commonwealth University, Dept of Forensic Science, 1015 Floyd Avenue, Rm 2015, Richmond, VA 23284*

After attending this presentation, attendees will understand how the quantitative and morphological analysis of 3D CT models can enhance facial approximation research by facilitating researcher-practitioner collaborations.

This presentation will impact the forensic science community by presenting new craniofacial relationships for the enhancement of facial approximation methods based on 3D CT skull and face models. In addition, new methods will be presented that facilitate comprehensive analysis, viewing, and sharing of 3D data.

Although facial approximation is described as a collaborative endeavor between anthropology, anatomy, and art, forensic artists have rarely been included in research. The exclusion of artists from research efforts has led to a lack of standardized protocols with many artists still using outdated standards and tissue depth tables. By including artists in craniofacial research, it can be ensured that the data being collected is relevant to facial approximation methods and encourage the incorporation of new data into facial approximation protocols. The use of 3D digital models generated from pre-existing CT scans allows the artists to have access to the valuable data they need: the simultaneous visualization of the craniofacial skeleton and facial features. These 3D models can be easily shared for morphological analyses and to gather input from artists to guide the collection of measurements by the researcher.

3D skull and face models were generated with the Mimics© software from publicly available de-identified head CT scans from The Cancer Imaging Archive at http://www.cancerimagingarchive.net. Bone and skin pixels were segmented at a threshold of 226 Hounsfield units and adjusted as needed to segment thin or less dense areas such as the maxillae and medial orbital wall. Instead of using the pre-programmed Optimal 3D reconstruction setting with contour interpolation, a custom setting was applied using gray value interpolation with no matrix or triangle reduction. This method results in more dimensionally accurate, high resolution models and also recovers features on superior and inferior surfaces that are normally truncated or stair-stepped with the optimal setting. Although the process is time-consuming, the generation of high quality 3D skull and face models allows the transfer of the Stereolithography (STL) models to external software for additional analyses and, more importantly, to the forensic artist for collaborative research.

The Simulation Module of Mimics© is used to place bone and skin landmarks to collect linear distances and angles. Morphological assessments are made using the free software tools 3D Slicer, MeshMetric, and MeshLab and has revealed many inconsistencies with facial approximation guidelines. Preliminary measurements indicate previously unrecognized bone-to-skin associations. For example, the distance between the left and right infraorbital foramina is significantly correlated with both the width of the mouth (r=0.48, p <0.01, n= 32) and the width of the nose (r=0.56, p <0.001, n=33). In addition, one individual with a bifid nasal spine was identified who has extremely high tissue depths for the entire subnasal/mouth region, suggesting that the identification of morphological indicators may be important for informing the application of appropriate metric data in facial approximation methods.

Because the exported skull and face models are in correct orientation to each other, a dense, objective facial tissue depth mapping method was developed using the publicly available software MeshLab. The face model is hollowed and cropped and then sampled against the skull using the Hausdorff distance filter. The tissue depth values can be saved in the vertex quality field of exportable Polygon (PLY) files representing the sampled face points and closest skull points. MeshLab filter scripts were also developed that allow for the separation of sampled face points and corresponding skull points into separate PLY files representing 1mm depth increments. All of the data generated for one head can be viewed simultaneously in MeshLab, including the 3D skull and face STL files, skull and face tissue depth PLY layers, as well as the 3D landmark coordinates collected from Mimics© (saved with the .xyz extension in a text editor). The ability to view all data in one free program greatly enhances the ability to exchange information and conduct more comprehensive analyses of craniofacial morphology.

Facial Approximation, Computed Tomography, Forensic Art

E83 Perception of Elder Abuse by Primary Health Care Professionals

Mafalda Ferreira, MSc, Faculty of Medicine, Rua Larga, 3004-504, Coimbra, PORTUGAL; César Santos, Delegação do Centro do INML,IP, Largo da Sé Nova, 3000-213 Coimbra, Coimbra, PORTUGAL; and Duarte Nuno Vieira, MSc, PhD, MD, Rua Antonio Jose de Almeida, No 117, Coimbra 3000-044, PORTUGAL*

After attending this presentation, attendees will better understand how primary care health care professionals perceive elder abuse and their role in preventing and diagnosing such cases.

This presentation will impact the forensic science community by illustrating the ongoing need to deepen health care professionals' knowledge about this issue and to provide them with tools to enhance diagnosis and treatment.

Elder abuse is currently highlighted as a public health problem in our society. Primary health care professionals are in a privileged position to recognize and manage cases of suspected elder abuse. The purpose of this study was to provide some insight as to how these professionals position themselves on this subject.

The methodology used in this research was a questionnaire elaborated and sent to physicians and nursing staff practicing in 12 different health care units in Coimbra, Portugal. The data collected included parameters related to demography, perception of abuse and management strategies, personal experience, and training on this subject. A 0.05 significance level was established.

The global response rate was 67.9%. There was a significant percentage of elders in the professionals' clinical files and most of them performed home care. Most (64.6%) considered abuse as more prevalent in the familiar context and 32.3% designated negligence as the most common type of abuse. Uncertainty in the diagnosis was one of the most important causes for non-reporting. Also, there were doubts concerning mandatory reporting of abuse to judicial authorities. Of the respondents, 87.4% stated they would feel more comfortable having a formal protocol to handle these cases. This subject was not included in the training curriculum of 70.9% of the respondents.

It was noted that there is significant contact between primary care professionals and the elderly population during clinical practice and home visits, which puts these professionals in an ideal position to access the elderly and detect signs of potential abuse.

Health care professionals also seemed to be aware of the relevance of elder abuse, as well as the importance of their role in preventing and diagnosing these abuses; however, a more extensive coverage of this subject during clinical training and the definition of general clinical guidelines favoring a multidisciplinary approach are important in increasing the professionals' confidence in managing suspected cases.

Elder Abuse, Elder Mistreatment, Primary Health Care

E84 A Comprehensive Comparison of Various Postmortem (PM) Fingerprint Recovery Techniques

Marzena H. Mulawka, MFS, John Jay College of Criminal Justice, 524 W 59th Street, New York, NY 10019; and Gary W. Reinecke, MA*, School of Medicine, Medical Campus, 72 E Concord Street, L1006, Boston, MA 02118*

After attending this presentation, attendees will understand the time, effort, personnel, supplies, cost, and issues involved in manual PM fingerprint recovery from Unidentified Human Remains (UHR) as compared to digital PM fingerprint recovery. The unpredictable condition of UHR resulting from various stages of decomposition is widely known throughout the forensic identification community. This unpredictability leads to various issues involved with recovering examination-quality fingerprints for forensic identification purposes.

This presentation will impact the forensic science community by comparing the time, effort, personnel, supplies, cost, and issues involved for various PM fingerprint recovery techniques. This presentation will provide results from a controlled experiment in an area with very little previous research and broaden the understanding of the different aspects of PM fingerprint recovery. A thorough understanding of the strengths and limitations of these techniques can enable Medical Examiner/Coroner's (ME/C) offices to better plan time management, resources, supplies, and personnel involved in PM fingerprint recovery for daily caseloads, as well as for Mass Fatality Incidents (MFIs).

Various methods for fingerprinting the deceased have significantly advanced and are continually progressing due to ongoing research, publications, and information sharing; however, even with the advancement of techniques, the manual recovery of PM fingerprints requires a significant amount of time, effort, personnel, supplies, and cost, especially when dealing with remains exhibiting significant PM changes. [1-8] Each case of PM fingerprint processing may require a unique combination of techniques due to the circumstances and environment surrounding an individual's death. Recovered remains may exhibit significantly compromised friction ridge skin depending on the severity of PM changes, such as rigor mortis, dehydration, decomposition, and animal/insect activity. As such, the condition of the friction ridge skin on each individual finger dictates which method must be used to successfully enhance and record any valuable friction ridge information. Multiple techniques may be used on each finger and the time and cost to employ them can vary considerably. Additionally, the forensic examiner may have only one chance to capture an examination-quality fingerprint record before a significant portion of the information is unsalvageable. Thus, the examiner should be properly educated and trained in the various PM fingerprint recovery techniques.[3,7]

Some ME/C offices and Law Enforcement Agencies (LEAs) have started to use digital livescan devices to capture fingerprints from the deceased. The benefits of PM digital fingerprint capture include immediate feedback on the quality of the fingerprint being obtained, as well as rapid response with results through various fingerprint databases. The development of mobile biometric devices could be used with single or multiple fatalities for the accelerated acquisition of fingerprint identification data with the potential for rapid identification. Previous studies of contact scanners reported that a full set of examination-quality fingerprints could be acquired in 45 to 90 seconds; however, fingerprints were only obtained from decedents where friction ridge detail was visible to the naked eye using contact scanners, but they could not be acquired from bodies affected by fire or showing advanced changes of decomposition. Furthermore, the devices had to be routinely decontaminated.[9,10]

The research discussed in this presentation explores the use of non-contact, 3D fingerprint scanners to evaluate their potential PM fingerprint capture. This research includes a series of fingerprint collections from taphonomically altered human remains, designed to mimic the types of cases that would be encountered in ME/C offices. Additionally, the research will benchmark non-contact 3D PM fingerprint recovery against existing manual and digital collection techniques in terms of quality of fingerprints and efficiency, as well as the time, personnel, and supplies necessary to process and record the fingerprints.[11]

The purpose of this presentation is to quantify, compare, and contrast all manual and digital PM fingerprint recovery techniques. Specific resources and supporting data will be provided for the time, resources, personnel, and costs involved for the various fingerprint recovery techniques currently available to the forensic community. In conclusion, streamlined PM fingerprint recovery techniques would be especially helpful for large agencies exhibiting a high caseload and during MFIs, when time and resources are significantly limited.[3]

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Unidentified Deceased, Postmortem Fingerprints, Fingerprint Recovery Technique

E85 Missing Persons: A Comparative Statistical Framework of the Phenomenon in Italy and the United States — To Identify Particular Characteristics and to Propose Improvements in Investigative Techniques

Patrizia Trapella, JD, MA, via Degli Artigiani 4, Este, Padova 35042, ITALY; Luca Massaro, MA*, via degli Artigiani n° 4, Este 35042, ITALY; and Matteo Borrini, PhD*, Liverpool John Moores University, RCEAP-School of Natural Science & Psych, Byrom Street, Liverpool L3 3AF, UNITED KINGDOM*

After attending this presentation, attendees will be informed about the main differences in the statistical framework of missing persons in Italy and the United States.

This presentation will impact the forensic science community by proposing ideas for improving the investigative techniques involved in searching for missing persons on the part of the forensic science community by multidisciplinary teams (sociologists, forensic anthropologists, victimologists, criminological, criminal profilers, suicide experts, police officers, and detectives).

In this field, it is very difficult to compare the actual state of knowledge between the United States and Italy. The two have very different cultural heritages, legislation, populations (United States: 328,900,000 in 2014; Italy: 60,795,612 in 2014), numbers of homicides (United States: 4.7:100,000; Italy: 0.9:100,00), and suicides (United States: 38,000/year; more than 3,000 in Italy). The numbers of missing persons also differ, being 84,924 between 1975 and 2014 in the United States and 29,234 since 1974 in Italy.

In spite of these differences, some findings are interesting. Data from the National Crime Information Center (NCIC) Missing Person and Unidentified Person Statistics and the Missing Persons Commissario Straordinario Reports highlight the fact that the main difference is that missing persons are classified according to age: the United States has only two groups — up to and including 18 years of age and those over 18 years of age — while Italy has three groups — up to and including 18 years of age, those aged 18 years to 65 years, and those over 65 years of age.[1]

There are basically five categories of missing persons: undetermined cause, voluntary absence, homicide, suicide, and accident. Searching for common characteristics among these categories is the basis for improving missing person investigations. But are these age-based subdivisions still valid? Are they affected by varying welfare policies? Are two or three groups useful or should there be more? Does reducing the number of groups make it simpler to seek or identify common factors? Why not categorize missing persons into age groups of 0-6 years, 6-18 years, 18-65 years, and 65+ years? Are man-tracking canines to be used only in cases of those aged 18 years or less and/or in those aged 65+ years; if so, why?

In Italy, approximately one-third of missing persons are foreigners. This is due to the mass immigrations into Italy during the past 20-30 years. In the United States, this differentiation does not appear to exist.

Within the concept of globalization, one relatively recent detail of the problem of missing persons, not reported in the analyses of the corresponding phenomenon in the United States, is the case of minors taken away from a non-Italian parent. In this case, the approach to the problem is not only investigative, but enters the field of international diplomacy regulating political relations between the countries involved.

In both the United States and Italy, the number of missing persons has remained substantially stable over the past few years. This indicates that a Missing Persons Task Force must be established to manage the thousands of still unresolved cases, primarily the older cases; otherwise these numbers will never significantly decrease.

With the goal of improving investigative work, this study suggests the need for an in-depth multidisciplinary study of the phenomenon involving suicide experts, criminal profilers, and victimologists, among others.

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Suicide, Missing Persons, Missing Person Investigation

E86 Determining Donor Gender Based on Blood Stains Using Raman Spectroscopy

Igor K. Lednev, PhD, University at Albany, State University of New York, 1400 Washington Avenue, Albany, NY 12222; Aliaksandra Sikirzhytskaya, MS, 1400 Washington Avenue, Albany, NY 12222; Vitali Sikirzhytski, MS, 1400 Washington Avenue, Albany, NY 12222; Ewelina Mistek, BS, University at Albany, 1400 Washington Avenue, Albany, NY 12222; and Lenka Halamkova, PhD, University at Albany, 1400 Washington Avenue, Albany, NY 12222*

After attending this presentation, attendees will better understand the recent advancement of this application of Raman spectroscopy. The implementation of advanced statistics for the analysis of spectroscopic data and the evaluation of the accuracy and reliability of the conclusions made will be discussed.

This presentation will impact the forensic science community by demonstrating the potential of the accuracy and effectiveness of biological stain analysis for forensic purposes.

Traces of body fluids discovered at a crime scene are a potential source of DNA, which is major individual evidence in the modern forensic investigation. The application of Raman spectroscopy for non-destructive, confirmatory identification of biological stains, including dry traces of sweat, vaginal fluid, semen, saliva, and blood, at a crime scene was recently reported.[1] This method allowed for differentiating animal and human blood as well menstrual and peripheral blood.[2,3] The theory behind Raman spectroscopy is based on the inelastic scattering of low-intensity, non-destructive laser light by a solid, liquid, or gas sample. Very little or no sample preparation is needed, and the required amount of material tested with a Raman microscope can be as low as several picograms or femtoliters. A typical Raman spectrum consists of several narrow bands and provides a unique vibrational signature of the material. Typically, non-resonance Raman spectroscopic measurements do not damage the sample. The stain could be tested the field and still be available for further use in the laboratory for DNA analysis. A portable Raman spectrometer is now a reality that should allow for this identification at the crime scene.

Men and women differ in many ways, including their chromosomal pattern, skeletal structure, and the average size of the stomach, kidneys, liver, appendix, and lungs. Women have three important physiological functions, which are totally absent in men, including menstruation, pregnancy, and lactation. These functions influence behavior and contribute to physical differences between men and women. It is most important for this study that the biochemical composition of blood is different for men and women. Women's blood contains 20% fewer red blood cells. A disparity in the coagulation factors and other proteins in plasma between the genders is well established. This study hypothesized that Raman spectra of blood might be sufficiently different between men and women so that a gender could be determined using this non-destructive analysis of a blood stain. Dry blood samples from a total of 60 donors were subjected to automatic Raman microscopic mapping followed by chemometrical analysis. Male and female blood spectral datasets were formed using MATLAB® 7.11 after preprocessing (baseline correction, noise reduction, and normalization by total area). Despite the fact that the average Raman spectra obtained for the two groups were similar, the unsupervised cluster analysis differentiated male and female blood samples satisfactorily. The most successful differentiation was achieved using the Support Vector Machine (SVM) algorithm followed by cross-validation by the sample-wise leave-one-out approach.

This project was supported by the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the Department of Justice.

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Gender Determination, Blood, Raman Spectroscopy

E87 Evaluation of Decomposition and Insect Colonization of Pig (*Sus Scrofa*) Cadavers Inside a Vehicle

Helene N. LeBlanc, PhD, 2000 Simcoe Street, N, Oshawa, ON L1H7K4, CANADA; Shari Forbes, PhD, University of Technology Sydney, PO Box 123, Broadway, NSW 2007, AUSTRALIA; Kelly Robinson, MSc, University of Ontario Institute of Technology, 2000 Simcoe Street, N, Oshawa, ON L1H 7K4, CANADA; and Alicia Buetter, BSc, Ontario Forensic Pathology Service, 25 Morton Shulman Avenue, Toronto, ON M3M 0B1, CANADA*

After attending this presentation, attendees will better understand the complexities involved in the assessment of remains recovered from the trunk of a vehicle; and how factors such as insect colonization delay, extreme temperatures, differing decomposition rates, and vehicle model must be considered.

This presentation will impact the forensic science community by illustrating how very few publications and little information regarding decomposition in a vehicle is available. This presentation will also include findings to enrich the forensic science community within this area such as explaining how the make of a vehicle can have an effect on insect colonization delay, as well as providing a clearer picture of decomposition in a closed vehicle environment.

Decomposition of a body in an open environment has been extensively studied; however, little is known of decomposition and insect succession within a closed environment, such as a vehicle. Decomposition in a closed environment will delay access of arthropods to the body, generate temperatures different than those in an open environment, and result in an altered rate of decomposition.

This study was conducted in southern Ontario, Canada, for two consecutive summers, using pig (*sus scrofa*) carcasses of similar biomass (25kg-27kg). Vehicles differed for each summer and specifically were a 1996 Volkswagen® Jetta® and a 1996 Pontiac® Sunfire®. For both experiments, the experimental carcass was placed in the trunk of the vehicle and a control carcass was positioned, unsheltered, on the ground 20 meters from the vehicle. Data loggers were placed inside the vehicles to record trunk and cabin temperature and humidity. In addition, a weather station was erected near the control carcass to record ambient temperature, humidity, and rainfall. To avoid introducing insects into the vehicle, sampling methods were developed to investigate the delay of insect colonization, pattern of insect succession, and general decomposition of the carcasses.

Results showed a delay of insect colonization of the pigs inside the vehicles of up to four days, as well as a greatly reduced species diversity. The elevated temperatures inside the trunk contributed to the rapid desiccation of the remains and, coupled with the accumulation of ammonia gas, resulted in the high mortality of insects. Despite using similar vehicles, differences in colonization delays were present on the experimental pigs, stressing that vehicle model should also be considered when conducting such a study.

Forensic Entomology, Vehicle, Colonization Delay

E88 Serial Killers in Colombia: A Comparative Study

Edwin O. Olaya Molina, BA, Diagonal 22B - 52 - 01, Bloque F, Bogotá 111321, COLOMBIA*

After attending this presentation, attendees will be aware of a brief comparative analysis of five serial killer cases in Colombia that occurred in the past 50 years and a recent special case that does not share the characteristics of others, a *sui generis* case.

This presentation will impact the forensic science community by showing, through comparative study of these cases, specific behavioral patterns shared by these murderers and how this becomes a snapshot of the phenomenon of serial killers in Colombia.

In Colombia, the names of Pedro Vicente Calderón, Pedro López, Daniel Camargo Barbosa, Luis Alfonso Garavito Cubillos, Manuel Octavio Bermudez, and Luis Gregorio Ramirez are not noteworthy for the vast majority, but if one speaks of the "Vampire bishop," "The strangler of the Andes," "The monster of cane fields," or the "Beast," memories and associations emerge more quickly. These men are recognized as murderers and in the literature are described as serial killers. Several have become old stories used to frighten children or horrific legends that are part of the criminal history of the country; however, not much is actually known about these men, their history, or all of the crimes they committed. The legal history of some of these murderers is lost in the archives or simply due to neglect. Fragments of these stories are recorded in one newspaper or another or in the memories of some judicial officers.

A thorough search for specifics about these murderers yielded information regarding their life stories, their murders, their *modus operandi*, the selection of victims, and how the murderers moved around the country. With this information, it was possible to conduct a comparative analysis of these serial killers. Thus, it was found that three of the men looked for male victims, children, two women, and especially girls. Four of these murderers showed a knack for winning the trust of their victims. Many of these victims lived in conditions of vulnerability due to financial or family factors. Several of these murderers killed not only in Colombia, but also moved to two and three other countries and committed crimes. All were caught, tried, and convicted. Some have since died, others are behind bars. One escaped from prison and today his whereabouts are unknown. The last of the serial murderers studied, who was recently captured and convicted, controlled several male victims by a sophisticated rope mechanism, and with this same rope system the victims took his life. The media dubbed this man "The monster of the ropes."

In conclusion, this study has identified the manner in which the serial murderer has appeared in Colombia, the *modus operandi*, the offenders' travel patterns, characteristics of the victims' vulnerability, and the difficulties in the identification and association of cases involving a serial killer.

Serial Killers, Comparative Study, Criminal Behavior

E89 Understanding Familial DNA Searching: Policies and Practices in the United States

Sara A. Debus-Sherrill, ICF International, 75 E Santa Clara Street, #300, San Jose, CA 95113; Michael B. Field, MS, ICF International, 9300 Lee Highway, Fairfax, VA 22031; and Saniya Seera, BA, ICF International, 9300 Lee Highway, Fairfax, VA 22031*

After attending this presentation, attendees will better understand the current landscape of practices related to familial DNA searching in the United States.

This presentation will impact the forensic science community by providing a comprehensive and systematic portrait of familial DNA searching in order to inform policy and practice. The field will be better equipped with the critical information needed to navigate the complexities related to this emerging practice.

Familial DNA searching is a forensic technique used to locate potential suspects through the identification of their family members' DNA in the Combined DNA Index System (CODIS). It has been used most extensively in the United Kingdom, but in recent years United States jurisdictions have expressed growing interest in adopting the practice. Proponents of familial DNA searching have cited its potential to facilitate the identification and conviction of suspects, prevent crime, resolve cold cases, exonerate wrongfully convicted individuals, and improve public safety; however, its use also raises important constitutional, ethical, and practical considerations for forensic scientists, criminal justice stakeholders, and policymakers.

Much of the information available regarding familial DNA searching currently stems from anecdotal accounts of its successes and scholarly critiques of the various constitutional, ethical, and practical concerns posed by legal scholars, advocacy groups, and criminal justice stakeholders; however, there has been little research on its use and perceptions about the practice in the field. With support from the National Institute of Justice, ICF International is conducting a mixed-methods study to produce a national portrait of familial DNA searching policies, practices, and legislation.

The study's multiple components include: (1) hosting expert roundtables of stakeholders representing diverse fields of expertise and perspectives related to familial DNA searching; (2) performing a literature review on familial DNA searching and related practices; (3) performing a legislative and administrative policy review on familial DNA searching and related practices; (4) administering a national survey of CODIS crime laboratories; (5) conducting intensive case studies in four states with varying practices related to familial DNA searching; and, (6) creating a cost-benefit framework to help guide jurisdictions in weighing their options regarding the use of familial DNA searching.

This study will present results from the National Survey of CODIS Laboratories and legislative/administrative policy reviews, including the extent of familial DNA searching among laboratories, policies and legislation guiding its use, diversity in specific practices, attitudes toward familial DNA searching and related practices, and challenges in conducting familial DNA searches. Implications for policy, practice, and future research will be discussed.

Familial, DNA, CODIS

E90 Statistical Analysis of Key Components of Alcohol-Related Sexual Assault in the Military

Michael J. Bosse, MFS, HQ, 19th MP Bn, CID, 314 Sasaoka Boulevard, WAAF, Schofield Barracks, HI 96857; and Unsil Lee, MS*, US Army Criminal Investigation Command, HI 96797*

After attending this presentation, attendees will understand the pervasiveness of alcohol as a contributing factor in sexual assault and the importance of applying a holistic approach when investigating such a complex crime as sexual assault, breaking down key components of sexual assault into the offender and victim demographics, and environmental and social factors combined with the proper identification, collection, and examination of physical evidence.

This presentation will impact the forensic science community by providing results from a statistical analysis of 317 sexual assault Reports Of Investigation (ROIs) investigated by four different geographically dispersed field offices of the United States Army Criminal Investigation Command (CID) over a two-year period (2012 and 2013), providing a broad perspective that forensically encompasses the identification, collection, preservation, and laboratory examination of physical evidence (i.e., DNA, fingerprints, or hair/fibers) as well as the analysis of intangible components of a crime.

Analysis of 317 sexual assault ROIs were categorized into sex (male versus female); alcohol-related versus non-alcohol-related sexual assault; four demographic age groups (18-25 years old, 26-34 years old, 35-49 years old, and 50 years and older); four different military rank groups (Enlisted Rank E1-E4, Enlisted Rank E5-E9, Officer Rank O1-O5, and Warrant Officer Rank WO or Civilian); and a monthly breakdown of ROIs (January through December, 2012 and 2013) in order to identify the age and rank groups of offenders and victims who were at the highest risk of being involved in alcohol-related sexual assault and to determine if seasonal climate changes were a contributing factor in the increase in alcohol-related sexual assault. This study used a quantitative analysis, using a contingency table analysis (the Chi-square analysis). It determined that the offenders and victims of all age and rank groups were in the likelihood of being involved in alcohol-related sexual assault with the p value greater than .05, except for the offender age group 50 years or older, ($X2(1, N=164)=4.01$, $p=.045$). Using the ratio comparison method, this study determined that 65% (207 of 317) of sexual assaults were alcohol related; 53% (109 of 207) alcohol-related sexual assault offenders and 72% (148 of 207) alcohol-related sexual assault victims were 25 years of age or younger (referred to as the target age group hereafter); 54% (112 of 207) alcohol-related sexual assault offenders and 56% (115 of 207) alcohol-related sexual assault victims were in the rank of E4 and below (referred to as the target rank group hereafter). Further, the analysis of the climatic factor confirmed a correlation between warm weather and the prevalence of alcohol-related sexual assault.

In conclusion, this study statistically substantiated that the offenders and victims of the target age and rank groups were at a higher risk of becoming an offender and/or a victim of alcohol-related sexual assault than those in all other age and rank groups. This study also substantiated that warm weather was a contributing factor in alcohol-related sexual assault. According to the military demographics prepared in 2012, service members who were 25 years old or younger made up 42.7% of the total active service member population (1,388,028). Well-thought-out alcohol-related sexual assault prevention policies and programs tailored to the target age and rank groups can potentially prevent the 42.7% of active duty population from becoming offenders or victims of sexual assault while enabling society to save an untold amount of resources (i.e., the laboratory expenses and/or investigative hours) associated with the conduct of sexual assault investigations.

Alcohol, Sexual Assault, Military

E91 Case Study of Postmortem Dismemberment Using a Coping Saw and a Related Analysis of the Cutting Plane Curvature

Eunah Joo, MS, National Forensic Service, Ipchunro 10 National Forensic Service, Forensic Safety Division, Wonju-si, Gangwon-do, SOUTH KOREA; Youngil Seo, MS, National Forensic Service, Ipchunro10, Wonju 220-170, SOUTH KOREA; Sangyoon Lee, MS, National Forensic Service, Ipchunro10, National Forensic Service, Wonju 220-170, SOUTH KOREA; Donghwan Kim, PhD, National Forensic Service, Ipchunro10, National Forensic Service, Wonju 220-170, SOUTH KOREA; Jin-Pyo Kim, PhD, 1524 Yusungdaero Yusung-gu, Daejeon, SOUTH KOREA; and Nam-Kyu Park, PhD, National Forensic Service, 10 Ipchoonro, Wonju, SOUTH KOREA*

After attending this presentation, attendees will better understand the correlations of the morphological characteristics of saw blades and the curvature of the cutting plane. Moreover, by analyzing a postmortem dismemberment case in which it was presumed that a coping saw was used, attendees will gain an understanding of the characteristics of saw marks from a coping saw.

This presentation will impact the forensic science community by suggesting new criteria for saw mark analyses. While few studies on the cutting plane shape can be found in the literature, the information from the curvature of the kerf wall can play a crucial role in identifying the tool that was used. Forensic scientists, especially those in charge of tool mark examinations, will have a reference for the size of the saw that was used derived from the degree of curvature of the kerf wall.

Saw marks remaining on bone indicate the features of the saw used for cutting. Because of the importance of identifying criminal tools, there have been steady advances in the analysis methods of saw marks; however, in contrast to well-established knowledge on saw types from the morphological characteristics of kerf wall striations and false starts, there have been few previous detailed studies on the overall shape of the cutting plane.[1-3] As is widely known, when the blade width of a saw is narrow, the cutting section of the bone forms a curved plane. Recently, more than 20 curved complete cuts were found in a postmortem dismemberment case, and it was presumed that the width of the saw blade used in the crime was unusually narrow. As a result of the examination, a coping saw was assumed to be used in the crime. This case motivated these efforts to study the correlation between the morphological features of saw blades and the curvature of the cutting plane. Generally, there are correlations between blade width, blade thickness, and kerf width with the curvature of the kerf wall. Therefore, measuring the curvature of the kerf wall can provide guidelines for presuming the size of a saw used in a crime scene.

The saw marks were cut into pig femurs, which have a hardness comparable to the hardness of human cortical bone.[4] Before cutting, the bones were cleaned by soaking in warm detergent solution and using an ultrasonic cleaner to minimize possible damage to the bone.[5] Saw marks were created with two types of hacksaws and two types of coping saws. Three tool mark experts cut the bones with a maximum tilt of the saw blade and selected the most curved cutting sections. An Alicona Infinite Focus 3D microscope was then used to measure the curvature of these cutting planes. The measured value of curvature of the cutting plane from the scene was compared to that produced in the laboratory. The curvature of the witness kerf wall was found to be much larger than the hacksaw-made plane. The upper limit of the criminal blade width could be set and excluded a considerable number of saw types from the list of suspect tools.

In this presentation, evidence will be presented indicating a coping saw was used as the cutting tool in this postmortem dismemberment case. The reliability of this new analysis method on the basis of the results of this experiment will be discussed. The results of the present study can be used to verify the type of saw used for cutting, in particular, in terms of the blade width and blade thickness.

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Dismemberment, Saw Mark, Bone Trauma, Curvature of the Kerf Wall

E92 A Proposal for a Universal Classification of Paraphilias

Anil Aggrawal, MD, Department of Forensic Medicine, Maulana Azad Medical College, Bahadur Shah Zafar Marg, New Delhi 110002, INDIA*

After attending this presentation, attendees will better understand the fact that paraphilias which have a wide range of activities from inflicting pain on one's self (masochism) to having sexual intercourse with the dead (necrophilia) can in fact be classified into a common universal pattern. Such a universal pattern of classification may help attendees gain insight into the range of different paraphilic behaviors, which can help practitioners compare and contrast the severity of different paraphilias across the entire spectrum of paraphilic patients. It is believed that ultimately this will also assist in developing some common strategies for treating different paraphilias and may help law enforcement understand the undercurrent of common patterns flowing through all paraphilias. This may have an important bearing on the medicolegal aspects of these paraphilias, for instance, in how each paraphilia has to be viewed and understood in relation to rest of the paraphilias.

This presentation will impact the forensic science community by stimulating further research into paraphilic behaviors and their classifications.

Paraphilic behaviors have existed since antiquity.[1] It has been shown that most paraphilic activities span a wide range of behaviors ranging from the most innocuous to the most deviant.[2] In 2010, it was shown that one of the paraphilias — necrophilia — could be organized into ten classifications. These classifications were named mathematically from Class I to Class X.[3] Class I necrophiles represents the most innocuous necrophilic behavior, which is comprised of role players. Role players only want their sexual partners to play dead while having intercourse. At the other extreme, Class X necrophiles or exclusive necrophiles are the most deviant type of necrophiles who commit murder in order to procure a dead body with which to have sexual intercourse. It has been conjectured and proposed that all paraphilic behaviors can be similarly classified into ten classifications.[4] In 2011, it was shown that all zoophiles could be similarly classified into ten classifications.[5] Recently, it has been reported that even non-contact paraphilias like exhibitionism can be classified in similar classifications; however, some proposed classifications among non-contact paraphilias have not been encountered so far.[6] It may be postulated that paraphiles belonging to such classifications do exist but have not yet been discovered. Such gaps may be viewed similar to gaps in Mendeleev's table of elements. It is hoped that just as gaps in Mendeleev's table helped stimulate a search for hitherto undiscovered elements, the current gaps would help discover thus far undescribed forms of different paraphilias.

Tentative names and defining characteristics for each classification are as follows: (1) Class I — role players who require the presence of a "consenting partner"; (2) Class II — romantic paraphiles who have a romantic relationship with the intended partner, which is not, or cannot be, reciprocated; (3) Class III — paraphilic fantasizers whose paraphilic tendency increases to masturbation/self-stimulation, but the target is still an image or an object; (4) Class IV — tactile paraphiles whose paraphilic tendency increases to touching the object of desire (the victim) or his sexual organs. In non-contact paraphilias like exhibitionism, this class is somewhat limited or modified. For instance, Class IV exhibitionists may want to touch the sexual organs in an image of a sexual object and may perform masturbation simultaneously; (5) Class V — fetishistic paraphiles are those who steal a fetishistic object from someone and keep it with them. They may display their organs to the fetish and masturbate; (6) Class VI — sadistic paraphiles whose paraphilic activity combines with torture/mutilation. In non-contact paraphilias like exhibitionism, true sadism or torture is not possible, since these are essentially non-contact paraphilias; however, this may involve an imagery of torture or actually whipping an image of a loved one; (7) Class VII — opportunistic paraphiles who are ordinarily content with normal sexual activity, but may resort to paraphilic activity if opportunity is available. If, by chance, they happen to face a lonely or unguarded victim, they may display their sexual organs. Typically, they are cowardly and docile and are content with just showing their genitals from a distance and masturbating; (8) Class VIII — classic/regular paraphiles whose paraphilic activity involves the classical and most commonly understood definition; (9) Class IX — paraphilic criminals whose paraphilic activity includes serious criminal activities, even homicide. In cases of non-contact paraphilias like exhibitionism, they also engage in other sexual crimes, especially pedophilia and child molestation. Upon finding a child alone, their sexual behavior may start with exhibitionism, but culminate with child molestation. These paraphiles are more dangerous to society; and, (10) Class X — exclusive paraphiles whose paraphilic activity is exclusively limited to a particular paraphilia with complete obliteration of any other form of sexual activity. In the case of non-contact paraphilias like exhibitionism, it is the *sole* outlet for sexual gratification. They cannot form a normal romantic relationship with a person of the opposite sex and cannot engage in normal sexual intercourse.

In conclusion, this presentation proposes that all paraphilias can be classified in similar gradations of ten classes ranging from the most innocuous to the most severe. This theory has previously been named the "theory of paraphilic equivalence." Such a holistic view of paraphilias would help understand their psychological and medicolegal aspects in a more comprehensive manner.

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Paraphilias, Forensic Psychology, Forensic Psychiatry

E93 An Interdisciplinary Approach to Forensic Science Education

John Mabry, JD, Forensic Science Institute, University of Central Oklahoma, 100 N University Drive, Edmond, OK 73034; Wayne D. Lord, PhD, University of Central Oklahoma, Forensic Science Institute, 100 N University Drive, Edmond, OK 73034; Mark R. McCoy, EdD, University of Central Oklahoma, Forensic Science Institute, 100 N University, Edmond, OK 73034; Thomas H. Jourdan, PhD, University of Central OK, Forensic Science Institute, 100 N University Drive, Campus Box 203, FSI Rm 110D, Edmond, OK 73034; and Dwight E. Adams, PhD, University of Central Oklahoma, Forensic Science Institute, 100 N University Drive, Edmond, OK 73034*

After attending this presentation, attendees will better understand a unique, innovative, and interdisciplinary approach to forensic science education.

This presentation will impact the forensic science community by illustrating a novel approach for forensic science educators.

Forensic science programs in higher education in the United States, at both the undergraduate and graduate levels, have traditionally been located in departments and colleges of universities offering degrees in the natural and physical sciences, although some programs can be found in social science departments such as criminal justice. Many of these programs are isolated within a specific academic department and this isolation can limit the depth and breadth of knowledge and skills obtained by students. Forensic science is truly an interdisciplinary field of study including specialties in pathology, engineering, odontology, toxicology, entomology, anthropology, psychiatry, psychology, biology, chemistry, computer science, and criminal justice. Isolating forensic science programs within one academic department can limit the options available to students seeking careers in forensic science and weaken the diversity of the pool of job candidates for forensic science laboratories.

This presentation describes an interdisciplinary approach at the University of Central Oklahoma (UCO) Forensic Science Institute (FSI) to educate future forensic science professionals. The FSI maintains that the UCO Forensic Science academic program, in particular with its interdisciplinary dimension, is unmatched in excellence, design, or vision by other forensic science academic programs found in the United States. After approximately 30 years, UCO has ceased to offer a stand-alone Bachelor of Science (BS) degree in forensic science. The forensic science baccalaureate academic program, formerly housed in the Department of Chemistry, now resides in the W. Roger Webb Forensic Science Institute, and has morphed into a unique configuration in which forensic science can only be taken as the companion in a concurrent degree program. All students completing a BS in Forensic Science must complete an additional degree. The BS in Forensic Science may be combined with any UCO undergraduate major with the exception of General Studies. Among the more common degree combinations selected by students are forensic science-biology, forensic science-chemistry, forensic science-computer science, forensic science-psychology, and forensic science-criminal justice. Accordingly, the forensic science degree can be awarded after a student completes the forensic science course requirements and earns either a concurrent degree or a second bachelor's degree. Research is at the core of the UCO FSI. A research-based thesis program was implemented for graduate students, undergraduate students were provided with research opportunities, and cooperative research efforts with the classical academic departments on campus were established with joint appointments held by forensic science faculty. This unique approach has proven successful in generating a diverse pool of job candidates to fulfill the ever-expanding needs of the forensic science work force.

Interdisciplinary, Education, Forensic

E94 Movement and Perception in Shooting Incidents: Neuroscience of Reaction and Reflex

Alexander Jason, BA, ANITE Group, PO Box 375, Pinole, CA 94564*

After attending this presentation, attendees will better understand the characteristics of human reaction and reflex responses in shooting incidents, how they differ, and how elements of neuroscience can be applicable to shooting incident analysis and reconstruction.

This presentation will impact the forensic science community by providing a basis for understanding the science involved within human performance in shooting incidents.

An officer-involved shooting incident analyzed by opposing parties in civil litigation involved issues in the timing of events, human perception, and response actions. One of the primary issues concerned the time interval between fired shots and the ability of a person to perceive a threat and to perform a responsive movement.

In a fatal, officer-involved shooting incident, the movement of the decedent's hand was an important issue. Two shots were fired by a police officer at a suspect, both striking the decedent's chest. The police officer described seeing the suspect's left hand in his sweatshirt pocket when he made the decision to shoot. The physical evidence showed that the decedent's left hand had been struck by one of the bullets (which then entered the chest), but the sweatshirt pocket was not perforated by a bullet. This apparent inconsistency in the police officer's description became the foundation of a claim that the police officer had been untruthful about the actions of the decedent.

Another key element in the plaintiff's case was that there was insufficient time between the two rapidly fired shots for the suspect to have moved his left hand out of his pocket after the first shot. There are known minimum time intervals for voluntary responses which require perception, mental processing, and motor program activation. The time interval for simple perception/reaction events can be very fast (average times are 0.20-0.25 seconds), and reflex actions can be much faster.

A comprehensive analysis of this shooting incident, which included timed movement studies and visual perception demonstrations, established that the suspect could have moved his hand from his pocket and up to his center chest after the first shot as a reflex action. This movement of the hand toward the central chest is a component of the human startle reaction and could have been performed in very short time interval, consistent with the interval between rapid gunshots.

An additional issue examined was what the police officer could have seen when he brought his pistol up to eye-level — and the time required for him to perceive, react (make a decision to shoot), initiate, and complete a motor program (pulling the trigger). This process requires a time interval in which the suspect could have moved his hand. All of these were important elements in the analysis of what could and could not have happened.

Shooting Incidents, Reconstruction, Neuroscience

JURISPRUDENCE

F1 The Principle of Guilt (Beyond All Reasonable Doubt) and the Presumption of Innocence in Italy: Juridical, Forensic, and Investigative Reflections on the Gallo Case

Patrizia Trapella, JD, MA, via Degli Artigiani 4, Este, Padova 35042, ITALY; Luca Massaro, MA*, via degli Artigiani n° 4, Este 35042, ITALY; and Matteo Borrini, PhD*, Liverpool John Moores University, RCEAP-School of Natural Science & Psych, Byrom Street, Liverpool L3 3AF, UNITED KINGDOM*

After attending this presentation, attendees will better understand an Italian case, covering a period from 1954 to 1961, which resulted in a sentence for murder even though no body was ever found.

This presentation will impact the forensic science community by describing a singular juridical error that took place in an Italian court. A study of the details of the case emphasize the requirement to integrate and coordinate investigations performed by various specialists attending crime scenes, in the hope that such integration and coordination will always take place, especially in cases of presumed homicide without a body.

The Case: Paolo Gallo, a farm worker, disappeared from a village in Sicily on the morning of October 6, 1954.[1] Bloodstains, his cap, two bloodstained stones, and a helmet with blood in it were found near the farmhouse where he worked. Crime scene investigators at the time calculated that approximately two liters of blood were found at the presumed crime scene. In spite of searching, no body was ever found.

Paolo's brothers Salvatore and Sebastiano were suspected of and subsequently condemned for the crime, although the trial was based only on circumstantial evidence: forensic evaluations of the blood turned out to be ambiguous; witnesses' statements were not checked and those statements which were believed to be reliable were, in fact, unreliable; and, a sufficient cause for the crime (i.e., motive) was not discovered. Above all, there was no body.

On October 7, 1961, Paolo Gallo was traced to the house where he lived, a few miles from the site of his "disappearance."

An analysis of the case revealed three critical points: (1) a poorly executed scientific test (hematology); (2) the intrinsic weakness of the circumstantial evidence; and, (3) the lack of any formulation or consideration of an alternative hypotheses. Italian law (Art. 533, Penal Procedure Code) states: "The judge shall pronounce a sentence of condemnation if the accused person turns out to be guilty of the crime in question, beyond all reasonable doubt." This disposition embodies a fundamental principle in the structure of juridical order: the need to seek the truth — not *of* the trial, but *within* the trial — by means of scientific methods.

How can the phrase "all reasonable doubt" be overcome in the case of a presumed homicide without a body? From the viewpoint of correct investigative techniques in cases of suspected homicide without a body, this study indicates the need to follow operating procedures, which comprise not only the continual involvement of expert teams (in particular, criminal profilers) but also mantracking dogs, and to be decided upon according to *preliminary* forensic and investigative presumptions, *sensu strictu*.

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Reasonable Doubt, Scientific Proof, Crime Scene Investigation

*Presenting Author

F2 An Analysis of Data on Wrongful Convictions on Grounds of False or Misleading Forensic Evidence (FMLFE)

Jude L. Jokwi, MA, Texas Southern University, 3100 Cleburne Street, Houston, TX 77004; and Ashraf Mozayani, PharmD, PhD*, Texas Southern University, 3100 Cleburne Avenue, Houston, TX 77004*

The goal of this study is to analyze the role that alleged false or misleading forensic evidence plays with regard to the overall population of wrongfully convicted individuals in the United States. This study also examines the demographic breakdown of the post-conviction exonerees on grounds of alleged invalid forensic evidence and further explores areas of policy enhancement to mitigate wrongful convictions on grounds of flawed forensic science.

This presentation will impact the forensic science community by educating forensic analysts of the human suffering and losses that result from human or technical errors in the analysis of forensic data. This presentation also explains the need for forensic analysts to consciously sustain an unbiased frame of mind throughout the chain of custody to analysis and presentation of forensic data at criminal trials in court.

Forensic science and its practitioners occupy an inextricable part of the criminal justice system. Given the nature of their jobs, forensic scientists are exposed to the possibility of making errors which could either be human or technical. These errors have at times unfortunately led to the exculpation of otherwise guilty suspects or to the wrongful conviction of innocent individuals. Even with all the safeguards that continue to be put in place to assure the quality of forensic evidence by the National Institute of Standards and Technology (NIST) and the Organization of Scientific Area committees (OSAC), forensic failings persist, even if minimal.

The ongoing drive nationwide for forensic laboratories to be fully accredited is an attempt by the forensic science community to mitigate the opportunities for errors in the analysis of forensic specimens. Though accreditation in itself is not the magic solution to the technical or human errors that occur during the forensic processes that generate forensic evidence, it reduces the opportunities for those errors to occur. Also, the need for better-qualified forensic scientists, the opportunity for peer review of procedures used, and analysis to produce clear and comprehensible laboratory reports have become more relevant to diminish error rates and error convictions.

This study uses secondary data from the National Registry of Exonerations (NRE) to qualitatively analyze the national incidence of wrongful convictions on FMLFE. The various categories of crimes and exonerees' demographic data will equally be considered. This presentation analyzes 367 exonerees on grounds of FMLFE culled from the NRE's list of all 1,617 known individuals who were exonerated between 1989 and March 2015 for various reasons. Generally, the themes that emerge from the data on exonerees from convictions based FMLFE are to a large extent consistent with those of the overall numbers in the criminal justice system.

Minorities are disproportionately overrepresented in the data of exonerees convicted on grounds of FMLFE, which is also consistent with the pain, frustration, and lack of trust in the criminal justice system prevalent in minority communities borne out in many studies. Of the 367 convicts exonerated on grounds of FMFLE, the race of 364 of them is known while that of three individuals is unknown. According to the NRE data, 48% of exonerees were White, 45% Black, 5% Hispanic, 0.6% Asian, and 0.8% represented undisclosed races.

Although it is always up to the trial judge to admit or reject forensic data in evidence, either decision impacts the trial outcome tremendously. Not everyone against whom forensic evidence is admitted gets convicted; however, the goal of the administration of justice is always to dispense justice to all parties. A vast majority of forensic examiners do their job properly, but given the potential for human or technical errors thwarting this goal, it becomes incumbent upon forensic science laboratories to be accredited and adopt unimpeachable ethical standards. This should be combined with scientific procedures acceptable to the scientific community that must be traceable, peer reviewed, and logically defensible. Also, forensic science laboratories should proactively adopt best practices as recommended by the American Bar Association (ABA) criminal justice section task force in 2012 requiring forensic scientists and analysts to be properly certified and for the results of their forensic analysis to be verified and comprehensively reported.

Misleading Forensic Evidence, Wrongful Conviction, Exoneration

F3 Ethical Responsibilities for Strengthening the Court System by Requiring a Basic Understanding of an Individual Forensic Science Discipline — The Judge, the Lawyers, and the Expert Witness

Joseph P. Bono, MA, PO Box 2509, Leesburg, VA 20177; Linda L. Chezem, JD*, Purdue University, 530 Denny Drive, Mooresville, IN 46158; Ted R. Hunt, JD*, Jackson County (Kansas City) Prosecutor's Office, 415 E 12th Street, Fl 11, Kansas City, MO 64106; and Betty Layne DesPortes, JD, MS*, Benjamin & DesPortes, PC, PO Box 2464, Richmond, VA 23218*

After attending this presentation, attendees will be able to better recognize the ethical and legal responsibilities of all parties involved in the courtroom to grasp the significant capabilities and limitations of the basic science related to what is being presented as expert witness testimony.

This presentation will impact the forensic science community by raising the level of awareness for judges, lawyers, and forensic scientists in understanding and, just as importantly, acknowledging inherent ethical responsibility related to expert witness testimony.

In "Daubert states" and in federal courts, the judge is required to serve as the gatekeeper in determining whether to admit expert witness testimony. The proponent of the expert witness testimony has the responsibility of arguing for the admission of expert witness testimony. The opposing party has a similar responsibility of arguing against expert witness testimony when they believe the testimony is irrelevant or unreliable. Are these responsibilities strictly ethical or do they cross over into legal requirements? Are there ethical responsibilities on the part of the judges to possess a basic knowledge of the science that is being introduced to the court and which they must "judge"? Do both advocates for the parties they represent have a corresponding ethical responsibility to understand and adhere to probable limits of the expert witness testimony they are introducing?

For the expert witness, are the sources of the expert witnesses' income relevant and under what conditions should a salaried person be required (much as consultants are required) to disclose pay and benefits for their testimony? Does the expert witness have an ethical responsibility to ensure that, no matter who is paying the bill, their testimony must be complete and unbiased by evaluating or at least acknowledging alternate explanations?

The adversarial nature of the courtroom will not, nor should it, change; however, is there a higher responsibility for all participants in the judicial process to adhere to an ethical code that requires more than a pro forma "keep it moving, attack without understanding the scientific basics" mentality whenever scientific testimony is introduced? Is the science really too complicated for the jurist? If so, is this a mitigating factor absolving lawyers and judges of an ethical responsibility to learn the basics of the discipline they are "judging," introducing, or countering? Does the forensic scientist have an ethical responsibility and a corresponding legal responsibility to ensure that unbiased standards for what is supposed to be opinion testimony are the primary guiding principles for rendering an opinion? If not, does the forensic scientist become a part of what those with alternative views are oftentimes accused of — operating on a win-at-any-cost mentality? Can scientific and legal ethics be taught at the university level to students who are preparing for a career as forensic scientists or are in law school? Is the current requirement for ethics instruction to obtain accreditation of the forensic science program adequate? If not, what is the alternative?

This panel session will bring together a judge, a prosecutor, a defense attorney, and a forensic scientist to present their views and to discuss how ethical requirements of those who testify as expert witnesses may be strengthened. This discussion will also include whether there should be ethical and legal requirements on the parts of all non-scientists to demonstrate a basic understanding of the science which they are responsible for admitting, presenting, or counter-arguing against in the courtroom.

Ethical Responsibilities, Expert Witness, Judges and Lawyers

F4 Scientific Evidence and the Law School Curriculum

Robert M. Sanger, JD, Sanger Swysen & Dunkle, 125 E De La Guerra Street, Ste 102, Santa Barbara, CA 93101*

The goals of this presentation are to: (1) demonstrate the ability to present a specialization certificate in the science program within the regular law school Juris Doctor (JD) curriculum; (2) explore the types of classes, the time and unit commitment of the students, and the demands on the faculty for such a program; and, (3) offer an approach for the American Academy of Forensic Sciences (AAFS) to provide a leadership role in establishing such programs.

This presentation will impact the forensic science community by educating law professors and administrators on the feasibility of a science and the law program within the requirements of a JD curriculum. It will also serve as a mild call to action to the AAFS to provide guidance and encouragement to law schools to develop such programs, to ultimately help guide their content, and to someday certify or accredit such programs.

Scientific evidence is increasingly prominent in the practice of law. The standard 15-week course on evidence dedicates approximately seven weeks to the hearsay rule and only two weeks to scientific evidence. In most law schools, there are a few courses on scientific evidence offered as electives, but there is rarely an actual scientific evidence curriculum. This presentation proposes that a specific, rigorous curriculum be offered with the intention of preparing law students for the real world of forensic evidence in the practice of law, particularly in the fields of civil and criminal litigation. The AAFS is a strong proponent of education in the forensic sciences. The AAFS maintains the Forensic Science Educations Programs Accreditation Commission (FEPAC), which provides goals and standards for forensic science programs at the undergraduate and graduate levels. This presentation suggests that the AAFS bring some of this commitment and expertise to bear on law school education as well as undergraduate and graduate science curricula.

Most law schools have a required curriculum for all, or most, of the first year and a number of required subjects that must be taken during the second and third years. Therefore, the opportunity to take elective classes is somewhat circumscribed. The primary focus of most law schools is to provide a general legal education that will enable the student after graduating to pass the bar examinations and to have a good understanding of the major areas of law, including torts, contracts, civil procedure, criminal procedure, Constitutional law, real property, corporations, wills and trusts, family law, and taxation. Students are encouraged to broaden their horizons by taking electives as time and schedules permit. A survey of law school curricula shows that the forensic science offerings are limited and are generally not presented as a coherent program throughout the course of study. Some law schools offer elective classes that pertain to certain subjects in forensic sciences, such as psychology and the law or computers and the law. Some offer a special class in scientific evidence; however, there are no law school programs for JD candidates that offer a specialization in elective study focusing on a general competence in forensic sciences and the law.

This presentation proposes that the AAFS assist in the creation of goals and standards for a science and the law curriculum for American law schools. This is not a program to educate forensic scientists nor a joint degree program. It is a part of the regular law school curriculum. The mission, goals, and implementation of a law school science curriculum are much different than those of undergraduate or graduate programs designed to educate students to become forensic scientists. The mission is to provide law students with a working knowledge of scientific principles, an overview of scientific evidence, and an opportunity to study one or two areas of forensic science in more detail. The goal is to prepare students to be better able to intelligently work with forensic experts. The long term goal is to give students the intellectual background to properly prepare experts to testify and to be able to challenge improper opinions proffered by opposing counsel.

This presentation also proposes that the AAFS consider adopting a certification, accreditation, or some other form of encouragement for law schools that demonstrate a commitment to the highest goals and standards of legal forensic science education. This can be developed over time and is in addition to the accreditation of law schools by the state bars, regional accreditation programs, the American Bar Association, or other agencies. If implemented, the encouragement of the AAFS would be of considerable value to the AAFS itself, the institutions, and the students who pursue the specialization certificate along with their JD.

Science Curriculum, Law School, JD

F5 Building Bridges Between Science and Law

Cynthia Blackwell, JD, Los Alamos National Laboratory, PO Box 1663, MS A187, Los Alamos, NM 87545*

After attending this presentation, attendees will understand the best practices and lessons learned from basic science education initiatives designed for the judicial and legal communities.

This presentation will impact the forensic science community by providing attendees with practical information that will assist in fostering successful science education initiatives and encourage participation in order to become part of the broader dialogue surrounding this issue.

In the wake of the 2009 National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward*, much has been written and discussed concerning the need to improve the science literacy of judges and attorneys, but little practical advice has been offered to assist in developing programs that achieve this goal. New Mexico is emerging as a leader in this endeavor through collaborations between the New Mexico judiciary, Los Alamos National Laboratory (LANL), the University of New Mexico (UNM) School of Law, and the National Courts and Science Institute (NCSI). This presentation describes the education process taking shape in New Mexico, offers attendees best practices and lessons learned from these initiatives, and provides a potential model for others to employ.

Utilizing a unique collaboration between scientists, judges, attorneys, and educators, a basic science course was developed for the New Mexico judiciary that allows judges to exchange their robes for lab coats. The goal of the five-day program is to provide participants with knowledge and experience that enhances their ability to evaluate the admissibility of scientific evidence. This is accomplished through a combination of interactive lectures, tours, hands-on laboratory exercises, and mock hearings designed around the following objectives: (1) understand and apply basic experimental methods common to all scientific problem solving; (2) explore and practice analytical methods used to interpret data and understand its limits; (3) examine data from personal scientific investigations and formulate conclusions; (4) present, argue, and qualify conclusions and inferences based upon scientific data; and, (5) discover connections in methods and language between law and science. The small class size and hands-on immersion aspect of the class are critical elements of its success. Judicial participants are extremely enthusiastic about the value of this educational experience and the course is now expanding to a national audience.

In addition, members of the same multidisciplinary development team and alumni judges are bringing science literacy to prospective new attorneys. A highly interactive course was developed for the UNM School of Law addressing the use of scientific experts in litigation with an emphasis on relating science fundamentals to their appropriate and ethical courtroom application. Skill-building lectures address such things as the scientific method, error and uncertainty, and jumping the analytical gap to draw conclusions from data. Students apply this knowledge through motion practice, mock depositions, and admissibility hearings. Feedback from students attending the course this spring was very positive with comments that it was the most valuable and practical course that they had taken in law school.

These two successful initiatives were the product of multidisciplinary teams generating a distinctive synergy that greatly enhanced the product and experience. Along the way, course developers and instructors gained a profoundly new understanding of one another, which is reflected in their materials and presentations. This collaborative approach can serve as a model to those struggling with implementing science education at the grass roots level. The key is to assemble a diverse group of committed practitioners, develop goals and objectives, and simply begin.

Judicial, Science, Education

F6 The Value of a Customer Working Group: A Customer's Perspective

Kristine Hamann, JD, 360 First Avenue, #10C, New York, NY 10010*

After attending this presentation, attendees will understand the benefits of having an ongoing relationship between senior members of a public forensic laboratory and senior members of its regular customers — the police and prosecutors. Such a Customer Working Group (CWG) can provide essential input to the laboratory on a variety of issues including backlogs, irregularities in the laboratory, and the implementation of new procedures and reports.

This presentation will impact the forensic science community by improving coordination between public forensic laboratories, police, and prosecutors so that backlogs, irregularities, and improvements can be discussed appropriately, efficiently, and quickly.

Forensic work is improving as scientific standards for forensic laboratories are evolving and becoming more rigorous. The demand for forensic work continues to increase and is spurring an increase in the number of forensic scientists and forensic disciplines. The boom in forensic science comes with new responsibilities and tasks, particularly for the laboratory director of a public forensic laboratory. Laboratory directors have to manage a diverse workforce, deal with budgetary constraints, and stay abreast of developing science, all while maintaining a quality system and dealing with growing backlogs based on requests from a criminal justice system hungering for more scientific evidence. Maintaining accreditation, improving efficiency, hiring and training scientists, as well as the responsibility of triaging of work to avoid backlogs, are all ongoing issues for a laboratory. In grappling with all of these matters, especially backlog concerns, laboratory directors will benefit from working closely with their regular customers.

A preliminary question is, "Who is the customer?" Though there are many stakeholders of a public forensic laboratory, including the criminal justice system as a whole, the funding authority, judges, law enforcement, the defense, and the agency to which the laboratory reports, these parties are not all regular customers of a laboratory. Police and prosecutors provide the vast majority of work to the laboratory and are the laboratory's regular customers. General guidelines for a laboratory's relationship with customers can be found in the International Organization for Standardization (ISO) 17025, Section 4.7. It emphasizes the value of ongoing communication and cooperation between the laboratory and the customer in order to clarify customer requests and to allow customers to monitor the laboratory's performance. Thus, it is important that the laboratory receives regular feedback from its regular customers — the police and prosecutors.

This presentation will outline the value of a CWG to a public forensic laboratory. The CWG can be instrumental in giving the laboratory director input on high-level policy issues of mutual concern, such as backlog reduction, how to respond to irregularities in the laboratory, the implementation of new forensic tests, problems with Laboratory Information Management Systems, and the updating of report-writing protocols. A CWG provides a forum through which its members can learn from each other, develop a consistent means of communication, and generate ideas for improving the laboratory.

Customer Working Group, Prosecutors, Police

F7 Asymmetric Politics and Forensic Science: "Forget It, Jake — It's Chinatown"

Max M. Houck, PhD, Forensic & Intelligence Services, LLC, St. Petersburg, FL 33705*

After attending this presentation, attendees will better understand how asymmetrical politics and information lead to adverse selection and moral hazards. The implications of dependent forensic laboratories to the justice system will be discussed.

This presentation will impact the forensic science community by explaining how asymmetrical politics and information lead to adverse selection and moral hazards and the "forensic failure" they can cause, such as wrongful convictions.

The vast majority of forensic service providers are governmental and operate in a political environment. Politics can trump science when it comes to operational decisions (policy, financial, perceptual). Forensic service providers may be subject to adverse outcomes because of political decisions.

As state agents, governmental forensic laboratories are dependent on their jurisdictional clients (police, prosecutors) for their raison d'être. Here, dependence means that one entity (dominant) can expand and be (more or less) self-sustaining while others (dependent) can do this only as a reflection of that expansion. An asymmetrical power relationship exists, with the subordinate entity having little or no ability to change and being relegated to a subordinate position. "In essence, if the rules of the game are biased against you, then you have two choices: to continue to play by the rules and thus continue to be exploited or to rewrite the rules in a way that does not leave you at an unfair disadvantage."[1]

Suggestions to rewrite the rules came from the National Research Council's (NRC's) National Academy of Sciences recommendation in its 2009 Report, *Strengthening Forensic Science in the United States: A Path Forward*:

> "Scientific...assessment conducted in forensic investigations should be independent of law enforcement efforts either to prosecute criminal suspects or even to determine whether a criminal act has indeed been committed. Administratively, this means that forensic scientists should function independently of law enforcement administrators."[2]

Suggesting is one thing, succeeding is another. Several jurisdictions have attempted independent forensic agencies with varying success. Power imbalances still exist in these attempts, leading to adverse selection and moral hazards. Adverse selection occurs when buyers and sellers have access to different information, leading to undesired results. If a bank set one price for all checking account customers, it loses money on both the low-balance and high-activity customers. Likewise, if attorneys demand that all cases are a "priority," then none are: all suffer equally. Moral hazards result when one party takes risks because they won't incur the potential cost of taking that risk. For example, when a prosecutor demands that evidence be tested even though experts from the dependent laboratory explain that the results will not provide useful information, then the prosecutor incurs a risk of resources on the laboratory. Unnecessary work needlessly consumes resources, wastes time, and delays the working of meaningful evidence.

Asymmetric information begins a downward spiral. Ultimately, market failure (the inefficient allocation of resources) may result. If other outcomes exist that improve one situation without worsening another, then market failure exists. Multiple scenarios for forensic science provision are possible, which would improve the quality and delivery of the science without adversely affecting justice outcomes. Arguably, the forensic industry is facing a type of market failure.

"Forget it, Jake; it's Chinatown." The last line from the famous movie, *Chinatown*, relates to the detective Jake Gittes. As a young police officer in Chinatown, Gittes once tried to protect a woman, but instead his efforts hurt her; this pattern is repeated in the movie's storyline. "Forget it, Jake; it's Chinatown" is an encouragement to Gittes to forget the current tragedy, just as he "forgot" the circumstances surrounding his time in Chinatown. Helpless to assist, Gittes sees how his intentions to do what was right were perverted by a corrupt and ineffective environment. Forensic science as Chinatown means that police and prosecutors will continue to bully laboratories, making them more susceptible to attacks by the defense. The profession becomes cynical about its future, as do stakeholders (police, lawyers, courts, media, the public). Punch drunk, laboratories will fail, services will falter, and the industry will stagger to its demise. Absent the political will to support forensic science sorting itself out independent of law enforcement and absent the intellectual infrastructure in forensic science to do the sorting, confidence in the profession will continue to be eroded. Failure, regrettably, is an option.

Reference(s):

1. Ishiyama J., Breuning M. 2010. 21st Century Political Science: A Reference. *Sage*. Page 330.
2. National Academy of Sciences National Research Council. 2009. Strengthening Forensic Science in the United States: A Path Forward. Washington, D.C. Page 22.

Market Failure, Wrongful Convictions, Independence

F8 Post-Conviction in the Wake of a Crime Lab Scandal: Lessons Learned From the St. Paul Police Department Crime Lab

Katie Conners, Office of the Public Defender, 2nd District, 101 E Fifth Street, Ste 1808, St. Paul, MN 55101*

After attending this presentation, attendees will understand how to approach post-conviction work after a crime lab scandal, including finding clients, prioritization of cases, arguments to present to the court, and strategic approaches to resolution of cases.

This presentation will impact the forensic science community by presenting approaches and responses when crime laboratories do not function properly and explain how those responses can make better science going forward.

First, attendees will learn what the initial response should be when a crime lab scandal emerges. This will include what the laboratory's ethical obligation is in notifying defendants of a potential post-conviction appeal, then how to effectuate that notification. Particularly for public defender offices in which the affected clients could be in the thousands, the outreach should be prioritized based on who is incarcerated and which cases are best situated for relief.

Second, after identifying and contacting clients, the next step is to start filing post-conviction motions for new trials. Depending on the state laws, there may be different approaches to theories under which the judge should grant a new trial; however, across the board, there is a *Brady* argument and a due process argument. In Minnesota, newly discovered evidence, manifest injustice for cases in which the client pleaded guilty, and false testimony in cases in which the client had a trial were also argued. In the alternative to these arguments, a fallback position of ineffective assistance of counsel was presented. Attendees will learn case law on these different theories of relief.

Finally, wins can come in many different forms, from the judge granting the motion for a new trial to renegotiating the case for less time to winning at trial. These different options should be examined in each case to determine how to get the best result for a client. There were primarily three counties in which the St. Paul Police Department Crime Lab processed cases. Whereas Washington County had a county attorney who was willing to negotiate sentences, Ramsey County and Dakota County did not. Attendees will learn varying strategies depending on what type of county attorney office is handling the cases and how to work together to achieve a just result that instills confidence in the system and confidence in the larger goal of better science going forward.

Appeals, Crime Labs, Exoneration

F9 Transferring Management of Forensic Operations From Police Department to Independent Non-Profit Corporation: Houston's Experience

Tom P. Allen, JD, Houston Forensic Science Center, 1200 Travis Street, 20th Fl, Houston, TX 77002*

After attending this presentation, attendees will understand options to address challenges resulting from the transfer of forensic operations from a police department to an independent, semi-autonomous, non-profit corporation.

This presentation will impact the forensic science community by detailing many of the challenges inherent in the transfer of forensic operations from a police department to an independent non-profit corporation, to prepare a transfer plan, and to address the concerns of stakeholders regarding the transition.

During much of the early 2000s, the Houston Police Department's (HPD's) Crime Laboratory was the subject of multiple investigations, employee terminations, civil lawsuits against the City of Houston, and, most importantly, exonerations of wrongfully convicted persons. Two HPD Chiefs of Police called for a halt to executions in cases related to DNA analyzed by the HPD Lab, and the *New York Times* speculated that the HPD Lab was the worst in the nation.

With the support of then-Mayor Bill White and the Houston City Council, the HPD ordered many changes that resulted in significant improvements in the Lab's operations; however, HPD executive management understood that true reform required a complete rethinking of the city's forensic operations. A series of confidential white papers pointed to what now appears to have been the only solution: HPD should not control what historically had been "its" crime lab. Instead, the Lab should be controlled by a separate organization, sponsored by — but still independent from — the City of Houston.

In 2011, Mayor Annise Parker instructed the city's legal department to investigate options for separating the HPD Crime Lab from the police department. The project brought together legal department attorneys, HPD police officers and civilians, and the Lab's new supervisors to work together (usually in agreement, but not always) to invent a non-profit entity to manage the city's forensic operations. In June 2012, the Houston City Council approved the creation of Houston Forensic Science Center, Inc. (HFSC), a local government corporation independently governed by a nine-member Board of Directors. Council's resolution also approved the corporation's certificate of formation, which includes a provision not typical of city-sponsored entities: although the corporation's Directors are appointed by the Mayor and confirmed by Council, no Director may be removed from office in the absence of a serious impropriety, as defined by the Texas Business Organizations Code.

In April 2014, pursuant to a closely negotiated interlocal agreement between the city and the corporation, HFSC assumed "responsibility for and control of" substantially all of the city's forensic operations, including the former HPD Crime Lab.

The transition from police department to independent, civilian management has presented multiple challenges. Examples include: (1) preparing organizational documents and subsequent agreements with the city that state obligations clearly but allow flexibility to respond to unanticipated circumstances; (2) ensuring genuine management reform while minimizing disruption of ongoing forensic operations; (3) enabling corporate management to direct day-to-day activities of all forensic personnel while allowing city employees to remain in the city's employ and to continue to participate in the city's retirement system; (4) enabling corporate (civilian) management to direct day-to-day activities of police officers; (5) melding a professional workforce consisting of non-unionized employees and members of two unions, often with different job titles, compensation, and benefits; (6) responding promptly to rapid changes in legal standards governing the use of scientific evidence in criminal proceedings; (7) developing and implementing a business model that will sustain the corporation without reliance on direct public funding; and, (8) responding to resistance to the concept of independent, civilian management of a forensic laboratory, including resistance from unexpected sources, such as the Federal Bureau of Investigation (FBI).

A thorough explanation of Houston's experience to date will assist public authorities and forensic professionals undertaking similar transitions.

Laboratory, Transfer From Police Control, Public Corporation

F10 Arc Burn: Not a Cause of Necrosis From Stun Gun Shock Wounds

James F. McNulty, Jr., JD, James Francis McNulty Jr. Attorney at Law, 915 S Calimesa Boulevard, Calimesa, CA 92320; Ismail M. Sebetan, MD, PhD*, National University, Forensic Sciences Program, 11255 N Torrey Pines Road, La Jolla, CA 92037-1011; and Paul Stein, PhD*, National University, Forensic Science Program, 11255 N Torrey Pines Road, La Jolla, CA 92037*

After attending this presentation, attendees will better understand the wounds caused by electrical shock at the points of percutaneous connection of dart-firing stun guns. This presentation discusses whether wound examination can be used as an investigative tool for determining whether a stun gun shock was discharged for an unexpectedly long duration and is, therefore, suspect of an excessive use of force.

This presentation will impact the forensic science community by finding that thromboses sufficient to cause necrosis is highly unlikely to present at the percutaneous connection of a stun gun shock of any realistic duration, even upon microscopic histopathological examination of biopsied and sectioned areas of the wound. Unlike arc connecting shocks, the wounds from percutaneously connected shocks are of no value when determining whether a stun gun shock was unexpectedly protracted and requires justifying explanation.

A single shock of 5s is the maximum duration of stun gun shock needed to subdue, even those who are violent.[1]

After this presentation, attendees will also appreciate that dart-firing stun gun shocks connect to their victim at two points, the points of electrical current entry and exit. At either point, the shock(s) may arc-connect to the victim at the skin surface or the dart connectors may imbed percutaneously. These shocks may cause enduring burn wounds. Surface wounds caused by arc connecting shocks of a duration ≥6s present with increasing amounts of visible necrosis, indicating the duration of the extended shock. At percutaneous connections, the shocks do not cause enduring surface burns but, instead, a simple puncture wound.

Whether arc burning contributes to the necrosis caused by extended stun gun shocks was experimentally investigated using a porcine animal model. The pig carcass was obtained from a local butcher. This study's premise is that thrombotic occlusion of blood vessels cannot cause coagulative necrosis of dead tissue; however, burn injury can still be observed after electrical arc contact. Shocks causing major electrical injury can also cause coagulation necrosis by one or both of two mechanisms. The shocks cause thrombi. Coagulation necrosis is an often encountered sequela. In cases of major electrical injury, arc connecting shocks cause arc burning of the flesh. A zone of coagulation is present at the central and most intense area of serious burns and this is the same location where the necrosis presents with burns from arc connected stun gun shocks.

The tissue above the pig's hooves was shocked with series of shocks of 3-, 6-, 9-, and 12-second durations at 7.18W from the circuitry of a formerly manufactured dart-firing stun gun, which is no longer manufactured. This circuit was selected because it operates at approximately twice the power of the dart-firing stun guns currently marketed and has much greater capacity to cause burns. Shocks of each duration were repeated three times to different areas of skin, once on each of three of the pig's feet. Gross examinations of the skin for wound development were conducted immediately after the shock with follow-up examinations at 30s, 1m, 5m, 30m, 1h, and 2h post shock. The skin showed no evidence of necrosis. Then three different areas of the skin were each shocked three times, once on each of the same three pig's feet with 30-second discharges from the stun gun circuit. The skin still had no grossly visible injury.

Experimental findings exclude arc burning as a cause of the necrosis. The findings also reveal that once a dart connector has imbedded percutaneously and breached the body's skin integument, current passes insufficiently in parallel circuit through the skin to otherwise cause coagulative necrosis of its tissue. This should remain the case, even when the wounding shock is extraordinarily protracted.

Reference(s):

1. Kornblum R.N., Reddy S.K. Effects of the Taser in Fatalities Involving Police Confrontation. *J Forensic Sci* 1991: 36(2):434-447.

Dart-Firing Stun Gun, Electroshock Weapon Wounds, Burn Wounds

F11 Forensic Science and Justice Integration — The Brazilian Experience: People and Systems Working Together for a Better Criminal Prosecution

Marcia Aiko Tsunoda, Msc, Departamento De Policia Federal, Sais Quadra 7 - Lote 23 - Setor Policial Sul, Instituto Nacional De Criminalistica, Brasilia, Distrito Federal 70610902, BRAZIL; and Jairo G. Schafer, MSc*, Justiça Federal, Rua Paschoal Apóstolo Pitsica, 4810, Florianópolis 88025-255, BRAZIL*

After attending this presentation, attendees will better understand a novel way of improving forensic science by analyzing the effectiveness of material evidence found by forensic scientists from the perspective of the final clients, the judges.

This presentation will impact the forensic science community by presenting the Brazilian experience of electronically integrating different criminal prosecution entities — an area with very little prior research in Brazil.

One can list many different ways to improve forensic science. New research, technologies, and methodologies are developed every day to address the challenges of reconstructing crime dynamics from different types of trace evidences left at crime scenes. New and improved equipment emerges to enable faster and more precise examinations; however, in this presentation, another way of improving forensic science will be addressed: improving the effectiveness of the material evidence found by forensic scientists based on feedback from the judicial system.

Since 2012, many workshops have been presented by Brazil's federal justice system in partnership with the federal forensic science body of the Brazilian Federal Police.[1] In these workshops, judges visit different forensic laboratories and engage in a question-and-answer session about the way forensic examinations are performed, scientific curiosities, and myths. The clarity and effectiveness of the forensic science report are usually discussed and many improvement possibilities emerge from such workshops.

Among all the cooperation initiatives that emerged from these events between forensic scientists and judges, the most successful was the computerized systems integration. In the federal justice's fourth region, there is a computerized system that manages all processes under their responsibility. On federal forensic scientists side, there is a national computerized system that manages all forensic reports and trace evidence chain of custody-related information. The integration between those systems allowed forensic scientists to understand how the forensic reports were being understood and utilized by judges by having direct access to the judgment, understanding what type of references are made in their forensic reports, then finding ways to improve the language, clarity, and precision of the reports.

The initial analysis of this system integration shows the importance of forensic science reports in forming the judges' convictions in the decision-making process. Some statistics from a one-by-one analysis are detailed here.

A detailed analysis was performed and statistics were collected from all environmental crimes judged in the years 2011 and 2012 for three Brazilian states. From 493 total reports, 149 were inserted in a formal criminal proceeding in the justice's system. In 115 of these 149 (77%), the forensic report's information was explicitly mentioned by the judges in their decisions. In 27 of these reports, the crime was defunct due to time elapsed or had been transferred from federal to state level. In six cases, the information was partially used by the judges and in only one case was the report of no help — the case was an oil spill that occurred at sea and the forensic scientists couldn't make a timely examination to ascertain the consequences.

This study also encompass the analysis of the impact of forensic science reports on counterfeit money, counterfeit documents, and handwriting-related crimes for the year 2011 in one Brazilian state. From a total of 520 forensic reports, 191 were inserted into formal criminal proceedings in the justice system. In 165 of these 191 reports (approximately 86%), the forensic report's information was explicitly mentioned by judges in their decisions. In 24, the crime was filed or had been transferred to the state's competence. In one case, the information was partially used by the judge and in one other case the report wasn't of any help.

Many forensic improvement projects started based on this feedback, breaking paradigms on how forensic scientists and judges can interact. Now, the procedures and recommendations regarding evidence examinations and reports can be upgraded based on the end user perspective. The feedback must be used as a mechanism for improving the organization.[2] For judges, as soon as the forensic reports are digitally signed, it will be possible to use the facilities of digital editing, allowing them, for instance, to use images, copy and paste functionality, and search for specific keywords. It is also important to note the increased speed in processing documents and the significant savings in mail and paper costs.

In conclusion, this case study demonstrates that, despite all the technology involved in forensic science dynamics, bringing together professionals who work for the same goal is an excellent way to optimize and improve results. Integrating people and systems proved to be a synergetic project that delivered surer justice to citizens.

Reference(s):

1. Braga M. Corregedora defende maior diálogo entre magistrados e peritos federais. Agência CNJ de Notícias. (Brasília, DF) 28 de agosto de 2012. In: http://www.cnj.jus.br/noticias/cnj/59098-corregedora-defende-maior-dialogo-entre-magistrados-e-peritos-federais
2. Möller C., Barlos J. Reclamação de cliente? Não tem melhor presente: Usando feedback do cliente como uma ferramenta estratégica. São Paulo: *Futura*, 1999, p. 102.

Integration With Justice, Computer System Integration, Feedback

F12 The Role of the English Coroner in Preventing Future Deaths in Similar Circumstances

A.R.W. Forrest, LLM, Office of HM Coroner, Unit 1, Gilbert Drive, Wyberton Fen, , Lincolnshire PE21 7TQ, UNITED KINGDOM*

After attending this presentation, attendees will gain an appreciation of how English statutory and case law have produced an environment in which the judicial inquiry into a death in an English Coroner's Court can lead to documented action to prevent future, and similar, fatalities.

This presentation will impact the forensic science community by showing that death investigation in a coronial system can lead to real benefits for the community beyond establishing the cause and manner of death.

English coroners investigate deaths by holding inquests when required by law. The process is expensive, time consuming, and may not provide all the answers the family of the deceased desire, since all the inquest record includes is the name of the deceased, cause of death, a brief description of cause of death, and a neutral conclusion.[1] How can this archaic process produce a lesson to help the living?[2]

In the past, juries were discouraged from adding riders to an inquest verdict which simply stated who the deceased was and when, where, and how the person died. Juries did still add riders, although they were not part of the verdict and were recorded in the margin of the inquisition; however, coroners had the discretion to record riders if it "might do a public good if it reaches a proper quarter."[3,4]

In 1936, the power of juries to attach a rider of blame to their verdict was abolished and the power to attach a rider of any sort was abolished in 1984. The coroner was then given permissive power to report matters (the continuation of which could be associated with future fatalities) to a person who could possibly do something about it.

In 2008, the coroner's position was strengthened by mandating that a report directed to the prevention of future fatalities after an inquest must be responded to within 56 days. The response had to indicate what action would be taken and if no action was to be taken, explain why not. A copy would be sent to the Lord Chancellor who would have the discretion to publish the report and the response. Making such a report was still a matter of discretion — or was it? The 1998 Human Rights Act incorporated the European Convention on Human Rights (ECHR), a post-World War II document written in 1950 in response to that war and the circumstances that led up to it, into English law. The ECHR thus became the nearest thing to a written constitution for the English that there is. Article 2 of the ECHR is the "right to life" part of the convention. Brussels Jurisprudence has held that Article 2 provides citizens an adjectival right to have their death "in the arms of the state" investigated and this investigation falls to the coroner, with an expanded remit to determine not only how the deceased died, but the circumstances in which he/she came to his/her death.[5] In a Court of Appeals case, there was *obiter* that in some inquests in which Article 2 of ECHR is engaged, the coroner had a duty to write a report in which the circumstances indicated that action might be taken to prevent future fatalities.

The Coroners and Justice Act 2009 requires the coroner to write reports that will be published, along with responses, when action may be taken to prevent future fatalities. This is a powerful tool for the doing good.

Examples of reports include those relating to notoriously dangerous roads, illicit cigarettes that are not self extinguishing, Novel Psychoactive Drugs which are not yet illicit, and the dangers of ignoring repeated chest infections, thus missing a diagnosis of Tuberculosis (TB), in patients being treated with tumor necrosis factor inhibitors.

The obligation in England and Wales for coroners to report deaths in which action may be taken to prevent future fatalities truly makes the Coroner's Court a place where the dead teach the living.

Reference(s):

1. The Coroners and Justice Act 2009, ss1-17.
2. Hawkes N. Why the delays in counting the dead? *BMJ*. 2014;349:g4305.
3. R v. Harding (1908), 1 Cr App Rep 219.
4. W B Purchase. *Jervis on Coroners, 8th Edition*, 1946; 110.
5. R .v HM Coroner for Western Somerset, Ex parte Middleton (2004) UKHL 10.

Coroners, Inquests, Learning Lessons

F13 Good Cop, Bad Cop — Forensic Pathology of Law Enforcement-Associated Deaths and Case Review

J.C. Upshaw Downs, MD, GBI ME, 925 A Mohawk Drive, Savannah, GA 31419; and Michael M. Baden, MD*, 15 W 53rd Street, #18B-C, New York, NY 10019*

After attending this presentation, attendees will better understand how medical examiners and consulting forensic pathologists scrutinize high-profile police-associated fatalities and how a systematic structured review system may improve the analysis of such cases.

This presentation will impact the forensic science community by suggesting possible methods to improve public confidence in final official conclusions in law enforcement-associated deaths while also enhancing ultimate case resolution in the justice system.

Among the most challenging of forensic science cases are those involving police agencies and use of force, especially when a victim dies during the interaction. Such cases then become high profile and are thrust into the media with intense scrutiny and opinions expressed. Recent months have seen a seemingly alarming rise in such cases, resulting in national attention and demands for reform as cases wind their way through the investigative process and into the justice system.

American penal reform was championed prior to the establishment of the United States by General James Oglethorpe (1696-1785). After a distinguished military career, Oglethorpe was elected to the British Parliament in 1722 where he championed penal reform (particularly for debtors) and humanitarian causes (anti-slavery, anti-impressment, and anti-alcohol), eventually creating the Colony of Georgia in 1733 with the objectives of philanthropy, military, and commerce. Indeed, the resulting city of Savannah was the first planned city in the present United States. The envisioned egalitarian community had certain core values at its inception: equal lands, banishment of slavery, fair trade with the indigenous population, prohibition of intoxicating liquors, and no lawyers. As so often happens, the best-laid plans went astray and Oglethorpe's vision of a promised land in which previously incarcerated debtors might receive a second chance went by the wayside as the opportunity attracted more middle-class settlers to Georgia. Most notably missing was the vision of equality for all under the law — civil and criminal equality is an ideal still sought in the present day. As such, Oglethorpe's desire was to be "'free from that pest and scourge of mankind called lawyers.' Oglethorpe and the Trustees (of Georgia) detested them, believing each colonist was capable of pleading his own case."[1] This seems an extreme non-sequitur, owing to the colloquialism, "A man who is his own lawyer has a fool for his client."

As recent police-related death cases have demonstrated, many in the population decry the lack of a classless utopian society — exhibiting a distrust of the police and doubting their ability to conduct impartial, unbiased investigations. Additionally, many jurisdictions utilize professional medical examiners employed by governmental entities, including law enforcement agencies. Small wonder there are those who doubt the "official version" of events once they are made public. In such situations, the only logical and reasonable recourse to redress grievances is to seek counsel from those with the skills and understanding to assist in such efforts. A structure allowing for open and thorough investigation of police-involved deaths, utilizing qualified and knowledgeable objective experts to scrutinize such cases could assist in resolving many questions in such matters and may provide the additional benefit of streamlining the flow of the case through "the system," eventually saving time and resources by anticipating potential pitfalls and concerns, thus restoring a sense of fairness.

Several examples of adverse outcomes in law enforcement-associated deaths are presented and discussed, including their eventual final resolution. These cases, reviewed from the perspective of expert forensic pathologists, are then analyzed in the context of a possible review system affording a multi-disciplinary assessment of the use of force and end result, with the ultimate goal of improving public confidence in the conclusions while enhancing both criminal and civil justice resolutions.

Reference(s):

1. http://www.visit-historic-savannah.com/savannah-history.html

Police, Deaths, Force

F14 The Baby Tyler Case: Should Medical Examiners Have Access to Statements Obtained by Law Enforcement to Determine Cause and Manner of Death?

Stephanie Domitrovich, JD, PhD, Sixth Judicial District of PA, Erie County Court House, 140 W 6th Street, Rm 223, Erie, PA 16501; Donald E. Shelton, JD, PhD*, University of Michigan-Dearborn, Criminal Justice Program, 4901 Evergreen Road, Dearborn, MI 48128-2406; and Jeffrey M. Jentzen, MD*, University of Michigan, 300 N Ingalls, NI2D19 - SPC 5452, Ann Arbor, MI 48109*

After attending this presentation, attendees will better understand the opposing views of judges and forensic pathologists as to whether medical examiners should be permitted to use the defendant's own statements to the police in their methodology or whether the medical examiner should instead adhere solely to reliance on objective, scientific, and/or medical evidence.

This presentation will impact the forensic science community by continuing the important dialogue among jurists and experts as to whether medical examiners should rely on defendant statements to law enforcement in the same way as physicians would rely on a patient's history in determining a diagnosis and treatment.

The recent decision of the Iowa Supreme Court in the case of State of Iowa v. Hillary Lee Tyler may shed light on the limitations of medical examiner testimony in criminal cases. In Tyler, the defendant was convicted of murder in the second degree for the death of her newborn son, Baby Tyler. The Iowa Supreme Court found the trial court abused its discretion in allowing the medical examiner to testify to the cause and manner of Baby Tyler's death and in admitting the unredacted autopsy report into evidence because the medical examiner based his opinions primarily, if not exclusively, on defendant's statements to the police as opposed to objective, scientific, or medical evidence. This case was remanded for a new trial.

After performing the autopsy examination, the medical examiner's expressed opinions on both the cause and manner of Baby Tyler's death was "undetermined"; however, in his final report, the medical examiner concluded the cause of death was "bathtub drowning" and the manner of death was "homicide." In forming his opinions, he relied on the defendant's statements to police. The medical examiner's report indicated: "The mother claimed she had given birth the previous day in the motel room and then placed the infant in a bathtub partially filled with water shortly after the birth. The baby reportedly moved and cried after birth."

Medical examiners routinely rely on defendants' statements provided to them by law enforcement to determine the cause and manner of death. Is this an improper comment on the defendant's credibility? Was the medical examiner's opinion on these matters based solely on scientific or medical knowledge, scientific standards, or technical training, or merely based on the medical examiner's adopting the statements and conclusions of law enforcement? Is the medical examiner's reliance on the defendant's statements to police the same as when a physician relies on a patient's history in reaching a medical diagnosis? Should the defendant's "right to vigorously and thoroughly cross-examine" affect the trial court's decision to admit or not admit this evidence for the jury's consideration?

Should medical examiners opine on cause and manner of death based on a combination of history including scene findings, witness statements, a combination of physical exams such as the autopsy findings, and then supplemental testing? What if the defendant's statements to the police were not credible and the product of coercion? What if the defendant's statements were not credible due to her medicated and vulnerable state? How does the medical examiner use this testimony? Would the medical examiner's report be an improper comment on the defendant's credibility?

While medical examiners are usually given the statutory responsibility of drawing conclusions as to the "cause and manner" of death, the conclusion as to "manner" of death is not necessarily admissible in a criminal prosecution. Assuming the medical examiner is properly qualified as an expert, there is no dispute that cause of death testimony based on an autopsy is ordinarily admissible because that is obviously a medical opinion based on medical examination; however, should trial judges as gatekeepers of the admissibility of scientific evidence permit medical examiners to go beyond their medical expertise and draw conclusions about the manner in which cause of death came about, based on information from the police or from a non-medical investigation? In this presentation, judges and a medical examiner will openly discuss this current topic.

Medical Experts, The Baby Tyler Case, Cognitive Bias

F15 The Role of the Forensic Pathologist in the Judge's Decision in Italy: A Presentation of a Peculiar Case

Anna Gitto, JD, University "Roma Tre" of Rome, Post-Graduate School of the Legal Professions, Via Ostiense, 159, Rome 00154, ITALY; Giovanni Serinelli, JD, University "Luiss - Guido Carli" of Rome, Post-Graduate School of the Legal Professions, Viale Pola, 12, Rome 00198, ITALY; Gabriella Fimiani, JD, University "Luiss - Guido Carli" of Rome, Post-Graduate School of the Legal Professions, Viale Pola, 12, Rome 00198, ITALY; Serenella Serinelli, MD, Sapienza University, Dept of Anatomy, Histology, Forensic Medicine, & Orthopedics, Viale Regina Elena 336, Rome, Lazio 00169, ITALY; Lorenzo Gitto, MD, Sapienza University, Dept of Anatomy, Histology, Forensic Medicine, & Orthopedics, Viale Regina Elena, 336, Rome 00169, ITALY; and Giorgio Bolino, MBBS, Viale Regina Elena, 336, Rome 00169, ITALY*

After attending this presentation, attendees will understand the principal phases of the Italian criminal procedure and the weight of the forensic pathologist's opinions in judges' decisions.

This presentation will impact the forensic science community by explaining the relationship between the forensic pathologist and the public prosecutor and their influence on the judge's pronouncement.

Italian criminal law is divided into two different parts: the Criminal Code (*Codice Penale*) that prescribes the types of crimes and the Criminal Procedure Code (*Codice di Procedura Penale*) that codifies rules describing every phase of the criminal proceedings.

When a violent death occurs, the prosecutor assigns an expert in the field ("*perito*" — a forensic pathologist in case of murders) to evaluate the case. The preliminary information reported by the forensic expert after the inspection will be used by the prosecutor to determine whether there is enough evidence to require a trial. If a trial occurs, there is the entrustment of the case (*conferimento dell'incarico*), a document containing questions that are asked of a forensic pathologist regarding the case. The prosecutor and the private parties can designate their own expert witnesses (respectively, "*consulente tecnico d'ufficio*" and "*consulente tecnico di parte*") who give support to their arguments. Once the trial has started, if the judge does not close the case with a non-suit judgment, he allows a hearing (*dibattimento*) based on the discussion among the parties. The respective results are extremely useful for the judge in coming to a decision.

The primary goal of this presentation is to present an emblematic case study of a homicide prosecution in Rome. A 28-year-old White male was walking down the street screaming incomprehensible phrases in a foreign language at night. At some point, a young man approached him and ordered him to stop screaming. After that, the man immediately started to assault the 28-year-old, beating him to death. According to the declaration of the witnesses, the man struck the victim with a kick to the chest, causing him to fall, then continued to kick the victim several times. The police and an ambulance were called. The rescuers tried to resuscitate the victim without success, and pronounced the death. The police notified the Public Prosecutor of the violent death; he then contacted the on-duty forensic pathologist, asking him to go to the crime scene. At the crime scene, the forensic pathologist saw the body of the victim placed on its right side on the ground between a motorbike (on the left) and a car (on the right), with the head toward the sidewalk (on the top) and the legs toward the center of the street, surrounded by a pool of blood. The next day, the forensic pathologist was requested to answer these questions after a complete autopsy: (1) cause of death and location of the injuries, their mechanisms, and the relative position between victim and offender; and, (2) the minimum number of blows, their nature (punches, kicks, others), and their severity in connection with the death.

At the postmortem examination, the head was found to be the main affected area. Bruises and lacerations were found on the skin of the face, while a linear fracture on the squamous part of the right temporal bone together with subarachnoid hemorrhage and brain contusions were observed. Toxicological analyses were negative. The death was due to intracranial injuries due to physical assault, and the manner of death was homicide.

Different types of homicide are described in Italian jurisdictions: (1) ''voluntary'' homicide is the killing of a human being in which the offender had intent to kill; (2) ''involuntary'' homicide is the involuntary action of causing the death of a person without criminal intent; and, (3) ''unintentional'' homicide occurs when the death of a human happens as a result of a deliberated and voluntary act of violence not meant to kill.

During the hearing, the judge took into consideration the outcomes of the expert survey of the prosecutor and of the private parties.

The forensic pathologist's opinion is usually decisive in court. Despite this, the judge is *peritus peritorum*, which means that he can freely and independently weigh all the results to reach a proper judgment.

Forensic Pathologist, Prosecutor, Judge

F16 Reporting and Presenting the Probative Value of Forensic Evidence in the Courtroom

Cedric Neumann, PhD, South Dakota State University, Mathematics & Statistics Dept, Brookings, SD 57007; Anjali A. Ranadive, JD, SciLawForensics, Ltd, 1834 Overlook Ridge Road, Brookings, SD 57006; Valerie Reyna, PhD, Cornell University, Dept of Human Ecology, B44, Martha Van Rensselaer Hall, Ithaca, NY 14853; and Graham Jackson, Advance Forensic Science, University of Abertay Dunee, 54 Craigie Hill Dumoig Leuchers, St. Andrews Fife, SCOTLAND*

After attending this presentation, attendees will better understand how best to present qualitative and quantitative data in court.

This presentation will impact the forensic science community by reviewing research on how data is understood.

Over the past two decades, the legal and scientific academic communities converged toward a consensus on the fairest and most balanced manner of reporting the probative value of forensic evidence. Both communities acknowledge that a trace recovered at a crime scene, on a victim, or on a suspect needs to be evaluated under two alternative propositions (one considering that the trace originated from a specific putative source (e.g., a suspect) and the other one considering that the trace came from some other source). The result of such evaluation is commonly reported as a "likelihood ratio."

There are several concerns often associated with likelihood ratios. The first concern is that courts do not necessarily share the sentiments of legal and scientific scholars and have repeatedly admitted scientifically unjustifiable forensic conclusions. The second concern is related to the ability of the forensic community to assign appropriate likelihood ratios for any type of evidence in any given case. During this presentation, there will be focus on a third concern: that of the presentation of likelihood ratios to lay individuals such that the probative value of the evidence is effectively understood and used in courts. Review of the jury study literature shows that researchers have mostly focused on descriptive studies of the jury's perception of heuristic conclusions (whether logical and justifiable, or not). Unfortunately, they have seldom attempted to build on psychological theories of human memory and reasoning to propose novel presentation methods, in particular for statistics as complex as likelihood ratios.

The objectives of this presentation are numerous. First, various heuristic methods currently used to report forensic evidence, from categorical statements to likelihood ratios, will be reviewed and their respective level of appropriateness and limitations will be discussed. Then, the foundations of a cognitive psychological theory that examines how humans remember, reason, and make decisions under uncertainty will be presented. It was found that, according to this theory, individuals remember in parallel the precise nature of information (e.g., a given number) and its essence (e.g., *a lot* vs. *small*). Individuals then use the least amount of information they believe is necessary to make their decisions. This theory not only explains how humans reason, but also illuminates reasoning fallacies and provides a basis to discuss, with the audience, the main findings of jury studies published to date. For instance, this theory enables us to understand the differences observed when potential jurors are presented with probabilities or relative frequencies, or when they are presented with likelihood ratios and error rates combined or separated. Finally, that theory will be drawn on to provide recommendations on how to best present and combine different aspects of forensic conclusions, such as similarity between trace and control samples, rarity of the trace characteristics in a population, probative value, and error rates.

Qualitative, Quantitative, Evidence

F17 A New Paradigm for Fingerprint Reporting … Without Individualization

Henry J. Swofford, MSFS, 4930 N 31st Street, Forest Park, GA 30297*

After attending this presentation, attendees will better understand the difficulties with supporting claims of single-source attribution (e.g., "individualization" or "identification") in the pattern and impression evidence disciplines, with specific emphasis on fingerprints, and be introduced to an alternative framework implemented within the Department of Defense.

This presentation will impact the forensic science community by exploring the evolution of fingerprint testimony as it pertains to "individualization" and "identification," highlight potential issues with the current reporting paradigm and use of such language, and recommend an alternative reporting framework without those terms to ensure fingerprint results are reported in an epistemologically compatible and more scientifically defensible manner.

For more than 100 years, fingerprint evidence has been used as a valuable tool for the criminal justice system. Relying on the generalized premise of "uniqueness," the forensic community has regarded fingerprint evidence as nearly infallible, having the capacity to "individualize" the source of a fingerprint impression to a single individual. While the uniqueness of a complete record of friction ridge skin detail is generally undisputed, the extension of that premise to partial and degraded impressions has become a central issue of debate. Nevertheless, forensic science laboratories routinely use the terms "individualization" and "identification" in technical reports and expert witness testimony to express an association of an item of evidence to a specific known source.

Over the last several years, there has been growing criticism among the scientific and legal communities regarding the use of such terms to express source associations which rely on expert interpretation. The crux of the criticism is that these terms imply absolute certainty and infallibility to the fact finder, which has not been demonstrated by available scientific data. As a result, several authoritative scientific organizations have recommended forensic science laboratories to not report or testify, directly or by implication, to a source attribution to the exclusion of all others in the world or to assert 100% infallibility and state conclusions in absolute terms when dealing with population issues. Consequently, the traditional paradigm of reporting latent fingerprint conclusions with absolute certainty to a single source has been challenged. The underlying basis for the challenge pertains to the mathematical logic applied during the interpretation of the evidence and the manner in which that evidence is articulated. By recognizing the subtle, yet non-trivial, differences in the mathematical logic, the fingerprint community may consider an alternative framework to report fingerprint evidence to ensure the certainties are not over or understated.

This presentation will: (1) discuss the logic largely subscribed to by the fingerprint community, along with the underlying basis as to why it is the focus of challenge; (2) present an alternative framework for the community to consider adopting, which is epistemologically more compatible and defensible; and, (3) discuss how this transition was achieved within the Department of Defense without minimizing the value of fingerprint evidence.

Disclaimer: The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the United States Department of the Army or United States Department of Defense.

Fingerprints, Identification, Individualization

F18 Progressive Forensic Exhibit Techniques for Court

Catyana R. Skory Falsetti, MFS, Maricopa County Attorney's Office, 301 W Jefferson Street, Phoenix, AZ 85003; Gary Hodges, BA, Maricopa County Attorney's Office, 301 W Jefferson Street, Phoenix, AZ 85003; and Dwayne Petray, BA, Maricopa County Attorney's Office, 301 W Jefferson Street, Phoenix, AZ 85003*

After attending this presentation, attendees will better understand how contemporary methods of forensic art and animation technologies are employed in court and how these methods can advance the clarity and absorption of case information.

This presentation will impact the forensic science community by providing examples of the techniques used in modern courtroom exhibits and the variety of types of models and images that go beyond the expected and beyond what has been used in the past. This presentation will introduce attendees to the possibilities of what can be accomplished in the courtroom when it comes to visual exhibits. The Maricopa County Attorney's Office of Phoenix, AZ, was forward thinking in the creation of a full-time civilian forensic exhibit specialist position in 1998. Because of an increased caseload and a desire to address client needs, this role expanded to three full-time forensic exhibit specialists in 2014. These individuals are available to all of the Maricopa County Attorney's Office attorneys for any of their exhibit needs.

A majority (60%-65%) of the human population are visual-spatial learners, by what is referred to as visual processing.[1] Research has demonstrated that juries have difficulty absorbing complex information delivered only in a verbal narrative fashion; however, information presented visually is much more easily understood and retained, with an even more significant increase in retention occurring when information is communicated both visually and orally.[2] Thus, having clear and effective visual exhibits are critical to making a persuasive argument.

Quality visual exhibits assist attorneys in understanding the complexity of their own cases, increase the power of pre-trial negotiations, help explain complex behaviors and facts to a jury, and provide anchor-point exhibits for the jury to reference during the course of a trial. Finally, exhibits can be complied into a "jury book" that can be used by individual jurors to make notes during trial and to reference later during deliberations.

Attorney's offices are often limited to the use of Microsoft® PowerPoint® to create presentations for court. More often than not, attorneys or paralegals with little to no experience with visual design are left to create important visual support for courtroom arguments, resulting in presentations that are not as effective as possible. Utilizing skilled professionals can lead to the creation of outstanding assets: compelling 3D illustrations, animations, and high-quality graphics.

Examples of the types of scenarios that Forensic Exhibit Specialists contribute to courtroom procedures in Maricopa County include digital image clarification and enhancement, examining video imagery, and selecting appropriate stills for use in identifying suspects. Specialists use all available software including the Adobe® Creative Suite, SketchUp®, Blender™, Cinema 4D, and others to create 2D and 3D crime scene presentations from crime scene drawings and Google® Earth imagery. The 3D animations of crime scenes can virtually take the jury through the crime scene without entering potentially pejorative imagery into court.

This study will demonstrate, via examples using commercially available software as well as proprietary technologies, how forensic artists contribute on a daily basis to the prosecution of criminal behavior in Maricopa County and how this skillset can be used elsewhere to create stronger courtroom presentations.

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2. Ferguson D. (The Hon Mr. Justice). 2004. The use of technology in the trial courts. *The Litigator;* Fall: 37-43.

Courtroom Exhibits, Animation, Forensic Art

F19 Forensic Metrology: An Important Branch of Forensic Science Toward Fair Justice

Veronica Scotti, JD, Studio Legale Scotti, via Emilia 160, Cadeo, PC 29010, ITALY; and Alessandro M. Ferrero, MSc, Politecnico di Milano, DEIB, Piazza Leonardo da Vinci 32, Milano, MI 20133, ITALY*

After attending this presentation, attendees will better understand the role of metrology in forensic sciences and how the most important concepts in metrology, such as measurement uncertainty, calibration, and traceability, help the trier of facts to better understand the results of scientific tests and render a decision beyond any reasonable doubt.

This presentation will impact the forensic science community by providing clearer insight into the basic concepts of metrology and show how the correct evaluation and expression of measurement uncertainty adds necessary and useful information about the probability that the value of the measured quantity (the measurand) lies inside a given confidence interval about the measured value. As a mathematical consequence, the probability (doubt) that the measurand value lies outside the give interval is also given. This presentation will broaden understanding among triers of facts of how a correctly presented measurement result, including measurement uncertainty, helps them quantify the doubt on how a correct decision is based on an experimental test.

Justice has always sought help in science to ascertain the factual truth. Experimental tests and measurements have become an important part of trials, especially since DNA profiling has been recognized as reliable evidence of someone's presence at the crime scene. The legal community considers science capable of providing fully certain answers, so in the past and in some countries even today, expert testimony expressing doubt is considered professional misconduct.[1]

However, the scientific community is aware that science cannot provide full certainty, since experimental (measurement) results cannot provide the exact value of the measurand, but only incomplete (and hence, useless) information about it.[2] Despite this, metrology shows how to turn this information into useful data by defining and evaluating measurement uncertainty. It is then possible to express a measurement result as a probabilistic interval of confidence about the measured value, within which the value of the measurand is supposed to lie with a given coverage probability.[2]

Uncertainty has recently started to be considered, although generally only in cases where the decision is based on values obtained through direct measurements, such as breath alcohol content or toxicological analysis.[1,3-5] In these cases, the instruments' contribution to measurement uncertainty, called instrumental uncertainty, is predominant and is the easiest to treat.[2] In important cases, namely fingerprint and DNA profiling, uncertainty also depends on another important contribution, originated by the inaccuracy of the employed method; in metrology this is called definitional uncertainty.[2]

Profiling is based on the identification of a number of common patterns in the samples to be profiled and in those belonging to known individuals. These patterns are the minutiae in fingerprint profiling and in the Short Tandem Repeat (STR) alleles in the DNA sequences.[6] Forensic science considers the probability of correct profiling given a number of corresponding patterns and therefore assesses the credibility of an identification on the basis of the number of patterns that have been recognized in both profiles.

The given probability represents, in metrology, only the definitional uncertainty; however, the contributions given by the employed instrument and the experience of the operator (instrumental uncertainty) also play a critical role, especially in DNA profiling. It will be shown that the actual probability of identification is the product of the probability of correct identification given the number of corresponding patterns multiplied by the probability of correct detection of the single patterns. Since this last probability may be, in many cases (latent prints, scarce or partially contaminated biological samples), significantly lower than the former one, uncertainty is much higher than that generally presented.

Two significant cases which occurred in Europe will be considered in which DNA profiling, absent uncertainty evaluation, could have led to a sentence of guilt for murder. The first case is that of bartender in Liverpool, United Kingdom, who was accused for murdering a young Italian woman during a robbery; the second case concerns the murder of a British student, Meredith Kercher, in Perugia, Italy, in which two students, American Amanda Knox and Italian Raffaele Sollecito, were first sentenced on the basis of DNA profiling, then released after measurement uncertainty was considered.

The proposed considerations and examples show how critical uncertainty is, and thus forensic metrology, in providing correct evidence to the trier of facts, and the legal and ethical issues that it poses to everyone involved in assessing the factual truth: judges, lawyers, criminalists, and expert witnesses.[7]

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3. Vosk T., Emery A.S. *Forensic metrology: Scientific measurement and inference for lawyers, judges and criminalists*. CRC Press, New York, NY, USA, 2015.
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Forensic Metrology, Measurement Uncertainty, Probability of Wrong Decision

F20 Better Ways to Manage Poorly Validated Scientific Evidence

Michael J. Saks, ASU College of Law, Box 877906, Tempe, AZ 85287-7906*

The goal of this presentation is to provide judges with a set of procedures to enable them to improve their ability to manage the admission of forensic science evidence.

This presentation will impact the forensic science community by helping to better prepare judges to evaluate proffers of expert testimony in the near term (while awaiting the reforms currently under way in the forensic identification sciences).

Numerous areas of forensic science are undergoing scrutiny and preparations for rehabilitation as never before. In the United States, most of that scrutiny is taking place within newly created administrative entities overseen by the Department Of Justice (DOJ) and the National Institute of Standards and Technology (NIST). The National Research Council (NRC) 2009 Report, *Strengthening Forensic Science in the United States: A Path Forward*, which launched all of those efforts, had recommended the creation of a National Institute of Forensic Science to undertake the reform work because it concluded that every institution that could have helped build more scientifically sound, dependable forensic disciplines had failed. In addition to crime laboratories, the larger forensic community, the larger scientific community, universities, the legal community, and government at all levels, the Report also faulted courts.

Although courts have had gatekeeping responsibility for expert evidence for several centuries (it did not begin with *Daubert*), they have yet to develop real proficiency in such screening. The NRC Report said that, "in a number of forensic science disciplines, forensic science professionals have yet to establish either the validity of their approach or the accuracy of their conclusions, *and the courts have been utterly ineffective in addressing this problem*." The clearest illustration of judges' failures to screen forensic expert evidence competently is the spectacle of courts welcoming into evidence forensic offerings that were later abolished once their lack of validity was exposed by scientific reviews external to the courts: voiceprints, comparative bullet lead analysis, and nearly two dozen arson indicators.

The reason for poor judicial performance in the gatekeeping of asserted science may be no more mysterious than that judges, as a group, lack the necessary knowledge and skill to evaluate empirical claims. They rarely insist upon seeing underlying evidence testing the validity of empirical claims, and few have the acumen to evaluate data that may be forthcoming. In a recent case, *Jackson v. Pollion* (2013), the eminent Judge Richard Posner excoriated lawyers and judges for their ineptitude regarding scientific issues.

With good reason, therefore, few observers expect judges to play much part in fixing the problems that plague forensic science. The current and ongoing assessments (and repairs) of forensic science are being conducted by more scientifically astute extra-judicial institutions. When those efforts bear fruit, judges will be able to harvest the bounty.

The question that judges can and should ask is: What can we do to better perform our responsibility to limit the risk that unvalidated or exaggerated expert claims will mislead fact finders? What can judges constructively do while waiting for new and improved forensic science? Here are some suggestions: (1) require examiners to be certified and their laboratories to be accredited; (2) require examiners to have participated in regular proficiency testing; (3) require laboratories to have submitted to routine scientific audits; (4) make use of court-appointed experts to reduce one-sided, low-quality, or exaggerated forensic evidence in the courtroom; (5) require blind examinations, evidence lineups, sequential unmasking, or other recognized procedures to minimize unintended bias in examinations; (6) employ partial admission: allow pattern-comparison examiners to describe similarities and differences between questioned and known samples, but not to opine on ultimate conclusions of identity; (7) require experts to stay within the bounds of their field's asserted expertise. The most that most courts could do in this regard is to require submission of relevant portions of major treatises in the field demonstrating that the specific task being performed in the case is one that the field believes it can do; (8) prohibit assertions of unique individualization (for fields that perform pattern comparison), assertions of perfect or near-perfect accuracy (for all fields), or specification of accuracy levels (or error rates) when those are known; (9) prohibit use of unreasonably overpowering terminology generally, such as "without doubt," "match," "share a common source," or "identification to the exclusion of all others in the world."; and, (10) instruct juries on the limitations of accuracy of particular techniques and types of expertise, even though the court has chosen to admit such testimony. This may be the most challenging of these ten suggestions, because it requires a judge to acquire some substantive knowledge of a field's limitations. Several reported cases provide examples of such instructions by the court.

Admissibility, Judicial Gatekeeping, Trial Evidence

F21 Upstream Remedies to Prevent Wrongful Convictions: Beating *Daubert* to the "Gate"

Peter Neufeld, JD, Innocence Project, 40 Worth Street, Ste 701, New York, NY 10013*

After attending this presentation, attendees will better understand proposed strategies for improving the transparency and scientific practice of forensic science.

This presentation will impact the forensic science community by sharing strategies for improving the transparency and scientific practice of forensic science through changes in national policy and laboratory practice.

In February 2013, the Department Of Justice (DOJ) and the National Institute of Standards and Technology (NIST) announced that the agencies entered a Memorandum Of Understanding (MOU) to create a National Commission on Forensic Science (NCFS) to set federal forensic science policy and the Organization of Scientific Area Committees (OSAC) to establish documentary standards in forensic science disciplines. The creation of these two agencies has spurred a national investment in forensic science. While the initiation of these bodies is an important follow-up to some of the recommendations of the National Academy of Sciences' 2009 Report, Strengthening Forensic Science in the United States: A Path Forward, additional national policy and laboratory practice changes are needed to improve the fragmented forensic science system in order to establish greater confidence in forensic science for all participants in the criminal justice system.

National policy changes that can support more transparent and reliable forensic science include: (1) A federal forensic science research agenda — a strategy needs to be established for forensic science research that incorporates the input of all federal agencies with an interest in forensic science as well as all federal science agencies that fund research. Research goals and questions should be set in advance to guide funding (more questions can develop as research studies advance) and agencies should oversee research funding in its areas of expertise; and, (2) setting standards for forensic processes AFTER research is complete. Many forensic science processes or components of forensic testing methods have been well established by rigorous, peer-reviewed research. Those processes should move forward for documentary standards setting at the OSAC. Processes in need of more scientific clarification must be thoroughly researched, then evaluated for measurement by NIST. Only after these two conditions have been met can the OSAC proceed with documentary standards setting. NIST has taken on the responsibility of validation research in forensic science and should set measurement standards for and evaluate the validity, reliability, and limits of forensic processes before documentary standards are set.

Laboratory practices that can support more transparent and reliable forensic science include: (1) testimony should be reviewed to meet the limits of science. Forensic science disciplines need to incorporate a more inclusive scientific community — such as researchers in adjacent areas of science and statisticians — and not just rely on general agreement between practitioners to establish testimony that reflects the current state and limitations of any forensic science process. Scientists should focus testimony on the testing documented in the reports and acknowledge that scientific statements made in testimony need to be supported by objective science. Last, the Federal Rules of Civil Procedure require that experts submit "Rule 26" reports that provide the experts' opinions, conclusions, and the bases thereof in advance of trial. Adopting "Rule 26" reports for criminal cases would establish a forensic scientist's opinion and limit the interference of attorneys in scientific conclusions; (2) definition of customer. A critique of the forensic science system is the perception of a lack of independence. One way that forensic science providers can demonstrate to criminal justice stakeholders that their mission is to provide independent scientific analyses to the system is to change the definition of the customer. In the United Kingdom, where crime laboratories also submit to the International Organization for Standardization (ISO) 17025 accreditation, the customer is defined as the courts in addition to the entity ordering the product or service. Redefining the definition of customer will empower forensic science providers to accommodate the needs of all forensic science end users; and, (3) reports and case files need to be transparent, comprehensive, and accessible. As impartial scientific work products, forensic laboratory reports and case files should provide a comprehensive communication of the test, results, and limits, and should be readily accessible. Understanding that the complement of laboratory material may be too voluminous for a report, if materials are to be included in the case file, they must be complete, organized, and the table of contents should be included in the report. Reports must also include a statement that, in order to fully understand the testing in this case, one needs the case file which is available upon request.

Policy, Transparency, Testimony

F22 Holding the Gate Open or Closing It: Evolving *Frye* and *Daubert* Approaches?

Donald E. Shelton, JD, PhD, University of Michigan-Dearborn, Criminal Justice Program, 4901 Evergreen Road, Dearborn, MI 48128-2406; and Stephanie Domitrovich, JD, PhD*, Sixth Judicial District of PA, Erie County Court House, 140 W 6th Street, Rm 223, Erie, PA 16501*

After attending this presentation, attendees will learn whether *Daubert* and *Frye* jurisdictions are evolving in handling scientific evidence after the 2009 National Academy of Sciences (NAS) Report, Strengthening Forensic Science in the United States: A Path Forward, and if not, why?

This presentation will impact the forensic science community by continuing the important dialogue among jurists and experts as to whether trial judges should be educated in the forensic sciences so that trial judges can be better gatekeepers in admitting more reliable scientific evidence.

After the NAS Report described the fatal flaws in previously admitted forensic science evidence, many jurists expected the federal courts and those states that had adopted *Daubert* standards to be the first to closely examine the reliability of such evidence. After all, the *Daubert* trilogy of cases has held that trial judges, as gatekeepers of admissible evidence, must evaluate the reliability of the scientific basis of expert opinions before allowing the jury to hear those opinions. In the nearly seven years since the NAS Report was released, most state courts that have adopted the *Daubert* standards, and even many federal courts bound by the *Daubert* standards, continue to routinely admit virtually any forensic science evidence offered by the prosecution. How? And why?

In state courts, where the predisposition to admit prosecution evidence by elected judges is the strongest, there are various devices that have been used to handle *Daubert* to allow such evidence. In some states, the trial courts have found that while their states may have adopted *Daubert*, their courts do not choose to follow the United States Supreme Court holding in the subsequent *Kumho* case which applied *Daubert* standards to *all* expert testimony. Trial judges then can find that the testimony offered by the prosecution is "technical" or "experiential" rather than scientific and that therefore *Daubert* does not apply.

Other courts that state they are applying *Daubert* standards nevertheless emphasize the old *Frye* "general acceptance" prong of the standards and find that the prosecution evidence is admissible basically because such evidence has always been admitted. These judicial opinions may also be characterized by a further mischaracterization of the *Frye* standard itself. Even under *Frye*, the test is general acceptability within the scientific community, not general acceptance by the judicial community. So such trial courts simply cite the pre-NAS Report cases that admitted such evidence and do not even hold *Daubert* hearings, such as *United States v. Crisp*.[1] Is this due process?

What about so-called pure *Frye* jurisdictions? How do trial judges' gatekeeping functions in *Frye* jurisdictions compare to those of other trial judges' gatekeeping functions in *Daubert* jurisdictions? Do recent cases show a shift of these *Frye* jurisdictions leaning toward *Daubert* conversions? How do trial judges in their gatekeeping role handle scientific evidence today, such as experts testifying as to the unreliability of eyewitness testimony based on empirical research? Is such expert testimony generally acceptable in *Frye* jurisdictions today?

Also, why is there a strong resistance to the reality of the findings in the NAS Report? First, the law is simply not structured to accept change. Legal training is based on the concept of precedent and *stare decisis*, which teach that the answer to present questions can always be found by looking to the past. The law is a search for certainty while science is a search for truth. Second, there may be a demonstrable pro-prosecution bias in many judges, stemming from a prosecution background or a desire to be known as "tough-on-crime" judges at their next elections or for their future appointments to the bench.

Reference(s):

1. *United States v. Crisp*, 324 F.3d 261 (4th Cir. 2003).

Admissibility, ***Daubert*****,** ***Frye***

F23 How the Trial Judge's Gatekeeping Function Can Be Better Utilized to Bar the Admission of Unreliable and Exaggerated Opinion Testimony From Traditional Forensic Science Disciplines

Andrew Sulner, MSFS, JD, Forensic Document Examinations, LLC, 220 E 57th Street, Ste 200, New York, NY 10022*

After attending this presentation, attendees will better understand the current case law and rules of evidence that govern the gatekeeper's obligation to exclude proffered opinion evidence that is exaggerated or unreliable. Attendees will learn about the factors relevant to assessing the reliability of expert testimony and the methods by which lawyers and judges can more effectively scrutinize, evaluate, and challenge proffered expert testimony.

This presentation will impact the forensic science community by illustrating how lawyers and judges can become more proactive and effective in challenging the reliability of proffered expert testimony and how the failure to challenge poor and marginal evidence from traditional forensic science disciplines can lead to fraudulent and exaggerated opinion testimony.

The 2009 National Academy of Sciences (NAS) Report, Strengthening Forensic Science in the United States: A Path Forward, recommended that the handling of forensic science evidence in court be vastly improved, emphasizing the need to challenge poor and marginal evidence from traditional forensic science disciplines and to prevent fraudulent and exaggerated opinion testimony. The Supreme Court's *Daubert* and *Kumho* decisions, together with Rule 702 of the Federal Rules of Evidence (FRE), vests trial judges with the responsibility of acting as gatekeepers to exclude unreliable expert testimony. The 1993 *Daubert* decision recognized that "vigorous cross-examination, presentation of contrary evidence, and careful instruction on the burden of proof are the traditional and appropriate means of attacking shaky but admissible evidence." In deciding whether or not to admit proffered expert testimony, FRE 702 (as amended in 2011) requires the trial court to ascertain "if: (1) the expert's scientific, technical, or other specialized knowledge will help the trier of fact to understand the evidence or determine a fact in issue; (2) the testimony is based on sufficient facts or data; (3) the testimony is the product of reliable principles and methods; and, (4) the expert has reliably applied the principles and methods to the facts of the case."

To improve the reliability of expert testimony, trial judges must become more proactive in evaluating the reliability of proffered expert testimony and more proficient in identifying circumstances when the reasoning used in forming an expert opinion is not properly grounded or illogical. Recognizing that the trial court's role as gatekeeper is not intended to supplant the adversarial system, a third-generation board-certified forensic document examiner and former state prosecutor will address the current case law and rules of evidence that govern the gatekeeper's function to distinguish between evidence that is shaky but admissible from that which is unreliable and inadmissible. This presentation will also illustrate how lawyers and judges can become more proactive and effective in challenging the reliability of proffered expert testimony.

Judicial Gatekeeping, FRE 702, *Daubert*

F24 An Examination of Scientific Expert Testimony: Transforming Evidence Presentation in the Courtroom

Shirley Marshall, Teesside University, Borough Road, Middlesbrough, Tees Valley TS1 3BA, UNITED KINGDOM; and Hannah Fawcett, PhD, Manchester Metropolitan University, Manchester, Greater Manchester M165GX, UNITED KINGDOM*

The goal of this presentation is to develop a comprehensive understanding of how expert witness evidence is both delivered and evaluated in the courtroom. This research examined three key issues: (1) how fiber and DNA evidence is presented in court; (2) how expert and non-expert evaluations of scientific evidence differ; and, (3) the effect that evidence presentation style has upon evidence comprehension and perceptions of expert witness credibility.

This presentation will impact the forensic science community by providing clear guidance regarding how forensic scientists can maximize their apparent credibility and enhance juror comprehension of scientific testimony.

Experts with suitable qualifications and experience testify in court on issues outside the everyday understanding of jurors. Their role is to assist the court in understanding the case evidence so a fair, informed verdict can be reached. Although experts can greatly aid juror comprehension, relatively little is known about how expert evidence is presented and evaluated in court. Research illustrates that jurors do not always reach unbiased decisions as stereotypes, preconceptions, and poor understanding of evidence accuracy have contributed to numerous false convictions.[1,2] It is therefore important to discover how best to present evidence in court in order to facilitate juror comprehension; however, there is insufficient research to establish whether jurors perceive scientists as accurate, clear, and compelling or whether they are overwhelmed by technical jargon and unconvincing presentation strategies. It would be beneficial to develop a comprehensive understanding of how expert witness evidence is both delivered and evaluated in the courtroom. Although psychological research has attempted to address this issue, the research on this topic is scarce. To this end, the current research examined three key issues: (1) how fiber and DNA evidence is presented in court; (2) how expert and non-expert evaluations of scientific evidence differ; and, (3) the effect that evidence presentation style has upon evidence comprehension and perceptions of expert witness credibility.

Twenty-three trainee forensic scientists (enrolled in BSc Forensic Science and BSc Forensic Biology programs at a university in northern England) testifying on blood (DNA) and fiber evidence collected in a mock hit-and-run incident were filmed presenting their findings in a mock court case. Their testimony was transcribed before being subjected to a detailed content analysis to ascertain their use of specialist terminology and the verbal and non-verbal features of the testimonies. Furthermore, an expert forensic scientist rated the accuracy, competency, and complexity of each individual testimony in order to provide an objective view of the trainee's abilities. Finally, mock juror participants rated the more subjective aspects of the testimonies, such as witness friendliness, attractiveness, evidence complexity, nervousness, and perceived expertise, of each trainee using the Witness Credibility Scale as well as returned verdicts in a mock trial utilizing the witness evidence.[3] Within this presentation, the effect upon the mock jurors of the different presentation techniques used by the trainees will be discussed. Relating the findings to past empirical research and theory, clear guidance will be given regarding how forensic scientists can maximize their apparent credibility and enhance juror comprehension.

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Expert Testimony, Juror Decision Making, Forensic Evidence

F25 From Crime Scene to Hipster Haven: Solving a Rape on Manhattan's Lower East Side

Melissa Mourges, JD, New York County District Attorney's Office, One Hogan Place, New York, NY 10013; and Martha Bashford, JD*, New York County District Attorney's Office, One Hogan Place, New York, NY 10013*

After attending this presentation, attendees will understand how cold case trials reflect changing crime patterns in gentrified neighborhoods.

This presentation will impact the forensic science community by illustrating cold case investigation and trial tactics and by showing how one suspect who was initially implicated by a "voice lineup" was exonerated by Restriction Fragment Length Polymorphism (RFLP) and a second suspect was implicated by newer DNA techniques.

Today, Manhattan's Lower East Side has the reputation for putting the "hip" in "hipster," but in the 1990s it was a neighborhood in transition; landlords who couldn't pay taxes abandoned tenement properties to squatters who lived without electricity, heat, and water. Throughout the 1990s, the 7th Precinct on the Lower East Side was among the top ten precincts citywide for homicides, cocaine use, and drug arrests.

In 1995, Susan A. was a 22-year-old college graduate from Maryland who made ends meet by working in a downtown bookstore. She lived in a third-floor apartment of a five-story brick walkup on Clinton Street on the Lower East Side, just two blocks from the police station.

As Susan came home from work late one April night, a huge man pushed in behind her as she put her key in the vestibule door. Shoving from behind, he threatened to kill her and pushed her up the stairs. He forced her to bring him to her apartment, but as they stood in the hall, he heard her roommates inside. He shoved her further upstairs to the rooftop, where he tied her scarf around her eyes as a blindfold, and sodomized her repeatedly. Then he put his hands around her throat and choked her to unconsciousness. When she regained consciousness, her nightmare had not ended; he turned her over and raped her, insisting she tell him how much she loved it. After rifling through her wallet, he ordered her to remain where she was, still blindfolded, for 20 minutes while he escaped.

Susan waited in fear until she was sure he was gone, then went home and called police. A rape kit was collected at the hospital, and Susan received assorted medications, to prevent sexually transmitted disease and pregnancy as well as the HIV "cocktail." Police asked Susan to view a suspect lineup in another 7th Precinct case, but she demurred as she had been blindfolded. Detectives conducted a "voice" lineup where suspects repeated threats the rapist had made. Susan identified a suspect, but RFLP testing by the Federal Bureau of Investigation (FBI) exonerated that man.

Susan's rape kit was tested years later as part of the New York Police Department (NYPD) Backlog Project, yielding a mixed male and female profile. The male profile hit to Tony Harrison in the Combined DNA Index System (CODIS); he was a predicate felon previously convicted of two robberies and a rape and sodomy. A second swab was taken from both Susan and the defendant; hers to compare to the female fraction to make sure the kit was intact and his to confirm the CODIS match.

The trial was comparable to a made-for-TV movie: the defendant, who had a long history of malingering, at 6'4" and weighing 300 pounds, appeared to fall into a dead faint during jury selection. An Emergency Room (ER) physician in the jury pool told the judge the defendant was faking it. When Emergency Medical Team (EMT) personnel hoisted him onto the gurney, he opened one eye, snagged a bag of cookies he had left on the defense table, and put them on the stretcher. After being "cured," the defendant refused to enter the courtroom. Every day the judge invited Mr. Harrison to attend his trial; every day he declined.

Jurors heard about RFLP and Short Tandem Repeat (STR) DNA testing, and a forensic pathologist who specialized in strangulation cases testified concerning the risk of death posed by the victim being choked to unconsciousness—recently, New York's legislature made choking a crime. Tony Harrison was convicted and sentenced to seven consecutive 25-year sentences. Unhappy with that result, he has made post-conviction motions alleging, among other things, that the "mixture" on the rape kit swab proved there was another assailant and that CODIS and laboratory officials violated confidentiality laws by informing prosecutors and police of the DNA match. Those motions have been denied; Harrison remains in prison.

DNA, Cold Case Rape, Gentrification

F26 We Don't Catch the Smart Ones — How a Rubber Glove Left Genetic Fingerprints at the Crime Scene

Rachel S. Singer, JD, Kings County District Attorney's Office, 350 Jay Street, Rm 1922, Brooklyn, NY 11201; and Diana Ho*, 421 E 26th Street, New York, NY 10016*

After attending this presentation, attendees will better understand the complexities of working on cold case investigations and how advanced DNA testing methods were helpful in developing evidence from a rubber glove and duct tape collected at an unsolved homicide.

This presentation will impact the forensic science community by illustrating the challenges in approaching a cold case investigation and the obstacles that both law enforcement and the forensic community frequently encounter. With the right resources, many of these cases can be re-opened and solved.

The murder occurred in the early morning hours of December 18, 2006, inside the victim's apartment in the Bathgate section of the Bronx as she was preparing to leave for work as a cook at a local children's daycare center. The 65-year-old woman was not discovered for two days, when anxious relatives had the fire department break down the door. She was found rolled up in a blanket in the back bedroom of her ransacked apartment. She was fully clothed with her head covered in duct tape. When the medical examiner unwrapped the body at the morgue, her arms were tightly bound behind her back with duct tape and her feet were taped together at the ankles. Clothing was tied as ligatures around her neck and torso. Cause of death was asphyxia caused by covering of the head and mouth. Decomposition from her body was so advanced the Office of the Chief Medical Examiner (OCME) was unable to develop her profile from her postmortem bloodstain card; it took nearly a year to develop a profile from her clavicle.

During the autopsy, the medical examiner found a piece of a yellow rubber glove lodged between the victim's arm and torso. That item, along with duct tape from the victim's body, was sent to the OCME for DNA analysis. Around that same time in 2006, the OCME began to use High Sensitivity (also know as Low Copy Number) DNA testing in homicide cases. The criminalists at the OCME developed a full male profile from the piece of rubber glove, which was then uploaded to the Combined DNA Index System (CODIS). The crime remained unsolved until 2008, when the laboratory received notification of a hit to convicted offender Mario Castro who was serving time for robbing and punching a young woman on the street who was returning to her apartment after work.

Despite the CODIS hit, there was more work to be accomplished. Based on signs of struggle and additional crime scene analysis, detectives believed more than one person was involved. The OCME tested duct tape from the victim's wrists and ankles as well as multiple suspect exemplars and elimination samples from family members who had worn clothing used as ligatures on her body. The laboratory was able to detect a mixture of DNA, including a different male than the cold hit suspect. After comparisons to the suspect exemplars and the application of the Forensic Statistical Tool (FST), a probabilistic genotyping system, the laboratory was able to provide results which helped identify an additional perpetrator, Pablo Garcia. Both defendants were convicted after trial and were sentenced to 25 years to life in prison.

LCN DNA, Cold Case, Forensic Statistical Tool

F27 Naked DNA: Mounting an Inadvertent Transfer Defense in Cases With Little or No Corroboration

Kelley Kulick, JD, Office of the Public Defender, 120 W Mission Street, San Jose, CA 95110*

After attending this presentation, attendees will have a working knowledge of primary, secondary, and tertiary transfer of DNA evidence and how this is a viable defense in criminal cases. Attendees will be provided a brief history of the research of inadvertent transfer, including documented cases of inadvertent transfer at crime scenes. Attendees will be given litigation strategies for defending inadvertent transfer cases with little or no corroborating evidence from investigation and motions through closing arguments.

This presentation will impact the forensic science community by offering practical litigation strategies for presenting a defense of inadvertent transfer in the courtroom where there is little or no corroborating evidence. This presentation will impact the broader forensic science community by elucidating the ongoing problems and issues surrounding the reality of inadvertent transfer in criminal cases. This presentation also serves to educate our justice partners about the serious risks inherent in the introduction of DNA match evidence into an investigation and into the courtroom when corroborating evidence is weak or non-existent.

Imagine that a man is arrested in his home for the murder of someone he has never met. The police interrogate him for hours and end the questioning by telling him his DNA was found on the victim's body. The man is adamant he is innocent. The science appears to be correct. As a defense attorney, where do you begin? Although we now have actual evidence of inadvertent transfer at crime scenes, defense attorneys do not understand how to prepare and present this defense to jurors who are not educated on this topic and judges that are apprehensive about admitting this evidence. A successful inadvertent transfer defense requires presentation of a cohesive theory from start to finish.

The preparation of an inadvertent transfer defense begins with proper investigation and the use of discovery to obtain necessary information. Attendees will also be taught to explore officer bias as a result of the DNA match through discovery and cross-examination. Motions *in limine* are the next line of defense, in which attendees will learn to utilize *Kelly-Frye* principles to preclude or limit criminalists from giving opinion testimony on probabilities of DNA transfer or identifying the method of transfer. After a discussion of preparation, the conversation will turn to presentation of the evidence in court to a jury.

This presentation will highlight that jurors want to know that inadvertent transfer is a reality and not a theoretical defense construct. Juror education on an inadvertent transfer defense will begin with thoughtful *voir dire* questions and the use of real-life examples in the cross-examination of the criminalists or direct examination of defense experts. Finally, education of the bench on this defense will include submission of jury instructions requiring corroboration of the DNA match to sustain a conviction. Overall, this presentation will provide solid case preparation materials on the defense of inadvertent DNA transfer.

DNA, Inadvertent Transfer, Litigation

F28 Disputed DNA Stats for a Low-Level Sample: A Case Study

Dan Krane, PhD, 3640 Colonel Glenn Highway, Dept Bio Sci, Dayton, OH 45435; Carrie Rowland, MSc, Wright State University, 2850 Presidential Drive, Ste 160, Fairborn, OH 45324; and Nathaniel D. Adams, BS, Wright State University, 3640 Colonel Glenn Highway, Dayton, OH 45435*

After attending this presentation, attendees will better understand the challenges associated with selecting a method for assigning statistical weights to a DNA sample involving dropout and an unknown number of contributors. Defense and prosecution arguments for and against a particular method of assigning a statistical weight in a specific court martial will be presented.

This presentation will impact the forensic science community by: (1) highlighting issues surrounding statistical weights in low-level samples; (2) describing incorrect but current practices in calculating statistical weights; and, (3) the arguments that were used to establish that a government laboratory's long-standing practice was unsound in this particular case.

The scenario surrounding the case in question involved an alleged sexual assault. A partial, low-level profile foreign to the victim was developed from a mixed sample taken from her underwear. The testing laboratory performed a modified Random Match Probability (mRMP) calculation on this "minimal minor" profile. While acknowledging that drop-out may have occurred and that it could not infer the number of contributors to the "minimal minor," the testing laboratory excluded as possible contributors all male genotypes considered in this case except for that of the suspect. A statistical weight of 1 in 220 was reported for the failure to exclude the suspect as a possible contributor.

The defense disputed the general acceptance of the testing laboratory's method of assigning a statistical weight and filed a motion *in limine* to exclude the laboratory's expert testimony on the matter. The defense argued that there is no generally accepted method for assigning a statistical weight to a mixed sample with an unknown number of contributors where drop-out may have occurred. The defense pointed out discrepancies between the description of the mRMP in the Scientific Working Group on DNA Analysis Methods' (SWGDAM) 2010 Short Tandem Repeat (STR) Interpretation Guidelines, the description of the mRMP in the testing laboratory's own Standard Operating Procedures, and the use of the mRMP calculation in the instant case.

The testing laboratory argued that its use of the constraints for using the mRMP described by SWGDAM were only recommendations rather than standards or guidelines and that it had independently "modified" the Random Match Probability to be used for samples with an unknown number of contributors where drop-out may have occurred. The testing laboratory further suggested that its accrediting agency, the American Society of Crime Laboratory Directors (ASCLD), had not found issue with its statistical approach.

The defense demonstrated that the laboratory had not independently developed a novel statistical method and that their calculations were identical to the mRMP formula described by SWGDAM.

The Judge Advocate granted the defense's motion, finding that the pertinent section of the SWGDAM guidelines "contains definitive, almost mandatory language," that the testing laboratory "used a statistical calculation in this case that does precisely what the Guidelines state is 'precluded,' " and that "a preponderance of the evidence does not indicate it is widely accepted in the field of forensic DNA testing despite…testimony to the contrary," and that "even if this Court were to determine… the resulting statistical calculations were reliable, the evidence fails the Military Rule of Evidence (MRE) 403 balancing test. The probative value is minimal." The Court's decision concludes, "the probative value is substantially outweighed by the danger of unfair prejudice, misleading the panel members, and a waste of time."

DNA, Statistics, Admissibility

F29 Overcoming Bias in DNA Mixture Interpretation

Mark W. Perlin, PhD, MD, Cybergenetics, 160 N Craig Street, Ste 210, Pittsburgh, PA 15213*

After attending this presentation, attendees will understand some principles of objective genotyping that help overcome cognitive and contextual bias in DNA mixture interpretation in order to provide unbiased match results.

This presentation will impact the forensic science community by showing how computer technology can resolve forensic questions in ways that remove unwanted bias.

Mixtures arise when two or more people contribute their DNA to an item of biological evidence. Unlike single source DNA data, there may be multiple possible genotype solutions for each contributor to the mixture. An inferred genotype (for a contributor at a locus) is therefore a list of allele pair possibilities, each with an associated probability.

Bias occurs when extraneous context or prejudice influence decisions. With DNA mixtures, bias shifts genotype probability away from the data-derived distribution toward a more preconceived outcome. This can happen in at least three ways. First, at the *data* level, some DNA interpretation methods modify the observed data. For example, a human analyst may initially code some data peaks as "real" and others as "artifact," while also scrubbing peak height quantities. This human filtering can be inaccurate, since uncertainty is best resolved by assigning probability to multiple outcomes, not by making arbitrary choices. Worse, if the analyst has some knowledge of a desired outcome (e.g., knows the suspect's genotype), then contextual bias may impair his/her classification decisions. Next, at the *genotype* level, some deconvolution methods strive for perfect mixture separation without genotype ambiguity. Here again probabilistic assignments would be a more scientific way to address uncertainty. If the analyst knows a desired solution, that knowledge can skew their conclusions. Finally, at the *match* level, there are statistical approaches that are inherently biased by knowledge of a suspect's genotype. With Combined Probability of Inclusion (CPI), the suspect's genotype is required to determine whether or not to use a locus statistically. With some Likelihood Ratio (LR) methods, the suspect's genotype must be considered when assessing the evidence.

Cross-examination can uncover such bias, thereby undermining the credibility of an expert and their conclusions. "Did you know the defendant's genotype during your analysis of the evidence?" "Doesn't knowing your customer's desired answer bias your decisions?" "Have any scientific studies demonstrated otherwise?"

The admissibility of biased DNA evidence can be legitimately challenged. Federal Rules of Evidence (FRE) 403 balances unfair prejudice against probative value. Assuming the genotype of a defendant when analyzing mixture evidence data is manifestly unfair and prejudicial to the defendant and may artificially inflate probative value. A judge may decide that a biased match statistic should not be admitted into evidence.

Cognitive science can help limit human bias in DNA interpretation. Approaches such as "sequential unmasking" intentionally restrict an analyst's access to case context and defendant information. But, in the computer age, why are people even involved in critical steps that can potentially introduce bias and why develop "expert" software that mimics biased human behavior? An unbiased approach would have a computer thoroughly examine DNA mixture data without knowing the defendant's genotype. This examination can be done by separating out the (probabilistic) genotypes of each contributor to a mixture. Only once that separation has been completed, and after the evidence genotypes have been recorded, should a comparison be made between two genotypes to calculate their match statistic.

There are practical ways to eliminate cognitive bias in forensic science. One is to replace subjective human methods with objective computing. Another is to avoid computer programs that examine evidence data alongside a suspect exemplar. Sophisticated Bayesian modeling offers a more neutral computing alternative.

Sources of bias that can be introduced when examining DNA mixture evidence will be presented. Understanding objective computer solutions will help the analyst present more neutral results in court. Additionally, recognizing bias will help trial attorneys better challenge skewed evidence.

DNA Mixture, Objective Genotyping, Cognitive Bias

F30 Limitations of Current DNA Testing: Information That May Not Be in Reports

Charlotte J. Word, PhD, PO Box 5207, Gaithersburg, MD 20882*

After attending this presentation, attendees will better understand: (1) other important information that may not be provided in laboratory final reports; (2) the limitations of current DNA testing results and conclusions; and, (3) additional questions that may be asked of analysts or additional information that may be available in the testing laboratory case file and technical manuals to aid in understanding the test results and their meaning.

This presentation will impact the forensic science community by providing information that may assist law enforcement, attorneys and their clients, judges, analysts, consultants, and the trier of fact in evaluating and understanding the limitations of current DNA testing and the laboratory final reports.

When forensic science testing is completed, the data are reviewed and interpreted, comparisons are made, and reports are issued. Generally, all reports provide some basic summary of the work performed and the conclusions reached; however, it is unlikely that even lengthy or detailed reports provide all the information that may be needed by all parties to understand what testing was actually performed and what the data obtained specifically mean in relation to the case being investigated and prosecuted. For example, reports often do not include information regarding negative tests, errors or re-testing that occurred, and whether any corrective actions were needed involving the case. Reports may also be incomplete such that all findings and all assumptions that were used during the testing and interpretation of the data may not be included. Furthermore, the language used in reports by different laboratories (or even departments within a laboratory) may have different meanings, leading to misunderstanding and misinterpretations by the users of the reports.

In addition to reviewing the information in the laboratory report, it is equally important to understand the limitations of the testing performed, the data obtained, and the conclusions reported. This is particularly true of DNA testing results that are issued from laboratories today. With the increase in recent years in the vast types of evidence samples routinely collected and submitted to DNA testing laboratories and the significant increase in the sensitivity of the Short Tandem Repeat (STR) testing assays, current DNA testing often results in incomplete mixed DNA profiles that are difficult to interpret. The reliability of the data and the relevance of the sample to the case may often come into question. Unlike the single-source DNA profiles obtained from visible biological stains in the early years of DNA testing in which essentially the identification of the source of the sample may be concluded and reported, many of the DNA test results being reported today are mixtures from three or more individuals (i.e., complex mixtures) for whom a very small number of cells and DNA were present. Prior methods for providing meaningful evaluations of the strength of the DNA evidence have been misused, while others are being developed and introduced in the court. Many different questions arise regarding the meaning and value of the DNA test results obtained from these types of samples.

To obtain information important to the case and to further assess the meaning of the data obtained, it may be necessary to review the laboratory case file and the laboratory standard operating procedures manuals. Critical questions should be asked of the analyst and all handlers of the evidence to assess the events that occurred while the evidence was processed at each step throughout the chain of custody and to learn of any problems that may have occurred during the testing. This presentation will focus on the issues outlined above, the additional information that may be needed in a case, and the questions to ask in order to understand the limitations of current DNA testing procedures and reporting.

DNA, Limitations, Reports

F31 Two Worlds Collide: The Perspective of the Forensic DNA Lab vs. the District Attorney's Office and the Impact of the Errors Reported in the Federal Bureau of Investigation (FBI) Population Data

Courtney Head, MS, Houston Forensic Science Center, 1200 Travis Street, 26th Fl, Houston, TX 77002; and Inger H. Chandler, JD, Harris County District Attorney's Office, 1201 Franklin Street, Ste 600, Houston, TX 77002*

After attending this presentation, attendees will better understand the impact the amended FBI allele frequencies are having on a large metropolitan forensic DNA laboratory and the county's district attorney's office. Attendees will also have a better idea of the challenges associated with the amended data and the potential implications for the judicial system.

This presentation will impact the forensic science community as well as the legal and forensic DNA communities by introducing the different vantage points of the forensic laboratory and the key players in the criminal justice system.

The forensic DNA community has been worried about the potential impact on casework resulting from the FBI's amendment to the 1999 and 2001 Short Tandem Repeat (STR) population data. Some of the forensic DNA community may have also been concerned about the retroactive impact on completed casework dating back 15 years. The FBI has been quick to explain how the errors were discovered and the potential statistical impact. The changes in allele frequencies had minimal statistical impact; on average, the FBI anticipated less than a two-fold difference. Therefore, the changes forensically were expected to be minimal. The Houston Forensic Science Center's (HFSC) Forensic Biology Section decided previously completed DNA reports would be amended with updated statistics upon request. The laboratory did not intend to recalculate statistics on all DNA cases that used the FBI's 1999 and 2001 allele frequencies for statistical calculations. Meanwhile, the Harris County District Attorney's Office (HCDAO) was reviewing the FBI's report and contemplating the ethical and legal obligations in light of the potentially favorable, newly discovered evidence.

In all criminal cases, prosecutors have a duty to seek out and disclose all exculpatory, impeaching, and mitigating information in the prosecution team's possession. This long-standing obligation was first recognized as a constitutional due process right in 1963 by the United States Supreme Court in *Brady v. Maryland*. The Texas Legislature codified and expanded this with the passage of the Michael Morton Act in 2013. It continues beyond the final disposition of a case, and broadens *Brady* by requiring the disclosure of favorable evidence regardless of its materiality or admissibility. In essence, this means that all favorable evidence must be disclosed without exception.

With these obligations in mind, HCDAO recognized that all pending and disposed DNA cases would have to be recalculated. Thus, even the slightest change in statistics favorable to the defendant would be subject to disclosure. HCDAO initially requested this work on more than 100 cases. This seemed time consuming, but achievable. It appeared these cases would be simple: enter the data into the PopStats™ software, then type the new, updated statistics into an amended report; however, HFSC analysts quickly learned many of the requests involved cases that had been analyzed under different versions of the Standard Operating Procedures (SOP), interpretation guidelines had changed, chemistry had changed, and the laboratory personnel who had done the initial testing were no longer employed by the laboratory. Each of these issues impacted the ability to quickly amend a report. In some situations, all the scenarios applied, further compounding the issue and the report's timeliness. Interpretative changes also impacted conclusions. In some cases, new guidelines meant inclusions and statistics had to be changed, making the profile or mixture unsuitable for comparison. These issues created a new challenge for the HCDAO. Now prosecutors faced not only the potential of favorable evidence on all disposed and pending cases resulting from the amended population statistics, but also HFSC's changes to conclusions due to the revised SOPs and data interpretation standards. It was quickly realized that the seemingly "minimal" impact of the FBI's database errors could reach all disposed DNA cases from guilty pleas to jury trials, from probation sentences to death sentences. HFSC and the HCDAO found themselves grappling with the daunting task of case identification, mass *Brady* notifications to defense attorneys and defendants, record retention, and data mining.

Amended Data, Statistics, FBI

F32 Justice for All, Oversight for Some? The Independent External Investigation Requirement of the Department of Justice's Paul Coverdell Forensic Science Improvement Grants Recipients

Jeffrey A. Benson, JD, BensonLaw Forensic Counsel, PO Box 1356, Minnetonka, MN 55345*

After attending this presentation, attendees will better understand the independent external investigation requirement of Coverdell Grant recipients and why this requirement is important to high-quality forensic science.

This presentation will impact the forensic science community by providing a framework to examine laboratory certification of oversight by independent external entities as required of all Coverdell Grant recipients and determine if such oversight is necessary and properly occurring.

The Department of Justice Paul Coverdell Forensic Science Improvement Grant Program (Coverdell Grant Program), upon successful application, provides funding to state and local governments to improve the quality and timeliness of forensic science and medical examiner services, to eliminate backlogs in the analysis of forensic science evidence, and to train, assist, and employ forensic laboratory personnel, as needed, to eliminate such a backlog.[1]

In the United States, 2004 was a crossroads in DNA collection and testing as the number of samples needing testing ballooned as a result of the 2001 authorization of the Patriot Act, which broadened the list of offenses that required DNA collection. In 2004, the Justice for All Act (2004 Act) simultaneously greatly increased the number of samples needing testing by adding all federal felony offenses to the list of offenses that required DNA sample collection and attempted to reverse the growing testing backlog by adding significant funding for laboratories. The 2004 Act amended the funding authorization process with an important addition — all applicants must certify that there is a government entity and an appropriate process in place to conduct external investigation into allegations of two kinds: (1) serious negligence; or, (2) misconduct substantially affecting the integrity of the forensic results.[2] In fiscal year 2005, nearly $14 million was awarded to forensic laboratories.

In December 2005, the Office of the Inspector General (OIG) evaluated the application and review process for the Coverdell Grant Program and found that the National Institute of Justice (NIJ) was not enforcing the external independent investigation requirement and recommended: (1) that the grant announcement explain the definition of an external independent investigation and provide examples; (2) naming the entity that will be conducting the investigations; and, (3) NIJ consider requesting verification from the entity that will be conducting the investigations.

In January 2008, the OIG audited the Office of Justice Programs (OJP) administration of the independent external investigation requirement. Again, the OIG found that the difficulties of this requirement continued. They found that one-third of the identified external independent investigation entities (referred to as entities) did not have the authority or the capability to conduct oversight. The OIG recommended that the grant application contain more detailed information about the oversight entity. Several years have passed since the 2008 audit and no additional audits by the OIG appear to have been performed.

This presentation will discuss what oversight means in the context of the Coverdell Grant Program and if that oversight is necessary when most laboratories are accredited by various accrediting bodies. Should the oversight provision be repealed? Is there meaningful oversight of forensic laboratories that receive Coverdell grants? What does the oversight look like and what types of entities are conducting the oversight? Have the OJP and NIJ been enforcing the oversight requirements?

Reference(s):

1. 42 USC §3797m Use of grants (2004).
2. 42 USC §3797k(4) Applications (2004).

Oversight, DNA, Coverdell

F33 Geographic Variability of Active Ingredients in Spice Within Alaska as an Indicator of Mechanisms of Distribution and Manufacture

Dakota W. Emery, 18548 Whirlaway Road, Eagle River, AK 99577; Christopher R. Iceman, PhD, University of Alaska Fairbanks, 900 Yukon Drive, Fairbanks, AK 99775; and Sarah Hayes, PhD, University of Alaska Fairbanks, 900 Yukon Drive, Fairbanks, AK 99775*

After attending this presentation, attendees will understand: (1) the active ingredients that are commonly present within the designer drug Spice, which is found in various regions of Alaska; (2) the discrepancies between labels on Spice packaging that state it does not contain illegal substances; (3) the disparities in the active ingredients in the same brand of Spice purchased from different geographical regions; and, (4) inconsistencies in product packaging that points to how Spice is being manufactured and distributed.

This presentation will impact the forensic science community by serving as a validation of methods to identify illegal synthetic cannabinoids in Spice, provide law enforcement agencies with insight as to how Spice is being sold through legal smoke shops and head shops, and how new legislation has been combating the distribution of Spice in Alaska.

Designer drugs, like the increasingly popular Spice, are psychoactive analogs of illegal substances with understudied health effects and have traditionally been sold in packages labeled "not for human consumption." Spice consists of an arbitrary mixture of ordinary herbs (e.g., vanilla, red clover, kratom) that are sprayed with psychoactive synthetic cannabinoid compounds.[1,2] Since molecular structures can be specifically altered to circumvent legislation, recent legislative efforts have focused on regulating packaging instead of individual molecules, which has effectively reduced the distribution of Spice through legal avenues, but has not eradicated their widespread use in Alaska.[3]

Multiple active ingredients have been identified in samples from various regions in Alaska by using methanol to extract synthetic cannabinoids from the herbal matrix and reconstitute the extraction with toluene and N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) before subjecting the sample to liquid injection Gas Chromatography coupled with Mass Spectroscopy (GC/MS). The chemical 1-pentyl-3-(1-naphthoyl)indole, a scheduled substance also known as JWH-018, has been identified as one of the active ingredients present in samples from Soldotna and Wasilla. Multiple other JWH synthetic cannabinoids have been identified in the samples, all of which are scheduled substances at the state and federal levels. Some samples contain up to seven psychoactive ingredients, and the composition of the sample may vary based on purchase location for the same brand.

This presentation will provide preliminary data to support future projects that can help legislators identify which ingredients within Spice are the most hazardous and should be fast-tracked to be banned. It is hypothesized that homogeneity in the active ingredient within shops or geographic region points to a smaller number of local sources, indicating regional or in-house production. Homogeneity within a brand across regions and heterogeneity between brands points to a remote distributor.

Reference(s):

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2. Ogata J., Uchiyama N., Kikura-Hanajiri R., Goda Y. DNA sequence analyses of blended herbal products including synthetic cannabinoids as designer drugs. *Forensic Science International*, Volume 227 , Issue 1 , 33 – 41.
3. Carroll F.I., Lewin A.H., Mascarella S.W., Seltzman H.H., Reddy P.A., Ann N.Y. Designer drugs: a medicinal chemistry perspective. *Acad Sci*. 2012 Feb; 1248:18-38.

Designer Drug, GC/MS, Spice

F34 Debating Death: Examining Capital Punishment Legislation More Than 40 Years After *Furman*

Christiana Burgess, BS, BA, 104 N Grand Fork Drive, Edmond, OK 73003*

After attending this presentation, attendees will better understand the variances in capital punishment legislation in the United States. Attendees will consider the extent to which the combination of differences in legislation and the discretion of sentencing authorities influences the probability that the courts will sentence individuals to death arbitrarily.

This presentation will impact the forensic science community by compelling attendees to consider how United States Supreme Court decisions have either positively or negatively affected capital punishment legislation since *Furman vs. Georgia* (1972). This presentation will encourage attendees to identify the strengths and weaknesses of this legislation in order to foster discussion regarding how to modify legislation in the future and to transform the atmosphere of capital punishment in the United States.

Since it first ruled on the issue in *Furman v. Georgia*, the United States Supreme Court has addressed repeatedly the process through which the government chooses to charge an individual with a capital offense, as well as the particular criterion that must be present in order for a jury to return a death sentence.

Prior to *Furman*, death sentencing in any given jurisdiction in the United States was characterized by a substantial number of death-eligible criminal offenses, a unitary trial system that combined the issues of guilt and punishment, and/or little or no guidance provided to jurors in regard to sentencing.

Subsequent to the ruling in *Furman*, which held that capital punishment violated the United States Constitution, between 1976 and 2008 the Supreme Court provided further guidance and instruction in regard to legislation of the death penalty. The results of these decisions included prohibiting mandatory death sentences and requiring bifurcated trials as well as necessitating guided discretion for juries during deliberation. In addition, the Court limited the opportunities for death sentences by specifying which crimes and what individuals are death-eligible. The purpose of these requirements and specifications was to minimize the potential for the courts to impose death sentences arbitrarily; however, the successfulness of these instructions in reducing arbitrary sentencing is debatable. Variances in capital punishment legislation combined with the sentencing authority's discretion have the potential to influence the probability that the courts will arbitrarily sentence an individual to death.

The sample for this research study included ten states, five with the largest death row populations and five with the smallest death row populations. This study examined the capital offenses and aggravating circumstances for each state as defined by their criminal or penal code. Among the ten states, there were eight categories of capital offenses with a total of 135 aggravating circumstances. Of those circumstances, 72 belonged to the states with the largest death row populations, and 63 belonged to the states with the smallest death row populations. When combined based on the particular elements of the statutes, the number of aggravating circumstances across the ten states was reduced to 60. Only nine of the combined factors appeared in legislation for more than 50% of the states, while 24 of the combined aggravating circumstances appear in statutes for only one state.

The results of this study suggest that the overall lack of consistency between states' capital punishment legislation and the sentencing authorities' discretion in evaluating aggravating circumstances increases the likelihood that the courts will arbitrarily sentence individuals to death.

The data tends to suggest that an excessive number of aggravating circumstances has the same potential to influence sentencing decisions as having no aggravating circumstances or aggravating circumstances that are overly broad and vague. The number of statutorily defined aggravating circumstances in a given jurisdiction does not necessarily correlate with the number of individuals sentenced to death in that state as there was no significant difference between the number of aggravating factors in the states with the smallest and largest death row populations. The failure of jurisdictions to provide statutory definitions for terms within its legislation leaves the jurors with discretion regarding interpretation, which can also create the potential for arbitrary sentencing.

Capital Punishment, Aggravating Circumstances, Legislation

F35 Innocent, Yet Still Incarcerated in Minnesota

Cynthia L. Evenson, JD, 306 W Superior Street, Ste 1400, Duluth, MN 55802*

After attending this presentation, attendees will understand that not every innocent person has been exonerated.

This presentation will impact the forensic science community by explaining why it is important for attorneys to educate judges regarding complex litigation issues and how attorneys can effectively use forensic experts in the presentation of a case. This presentation will describe the outcome of a case in Minnesota in which the defense attorneys firmly believed in their client's innocence. After being found guilty by a jury, the case was referred to the Innocence Project, who assisted in attempting to exonerate the client. After these great efforts, the client is unfortunately still incarcerated.

In the summer of 1996, law enforcement responded to an address due to a report of a dead body inside the apartment. The decomposed body of a female was found lying on a bed covered by a blanket in one of the bedrooms. Her body and the surrounding area were covered with insect larvae in various stages of development. After a long investigation, the defendant was arrested 16 months later. He was originally charged by complaint, but a grand jury was convened three months after his arrest and returned an indictment for one count of first degree murder committed during domestic abuse.

There were two primary suspects in the case: the defendant, who was romantically involved with the victim and who had a history of assaulting her, and the victim's former female lover, F.W., who had also previously assaulted the victim. The defense brought a motion and offer of proof to admit alternative perpetrator evidence that the former lover, F.W., committed the crime. The court only allowed the defense to present 33 of the 68 offers of proof to the jury in support of its alternative perpetrator theory of defense. One of the critical items excluded by the court was that F.W. had subsequently killed a male in a manner very similar to that of the victim in this case.

The state called three witnesses who testified that the defendant made admissions: two were very weak admissions by the defendant and the third was a jailhouse inmate who received a benefit in the case he had pending in exchange for his testimony. The defense called several witnesses to testify that F.W. had made admissions of killing the victim. The defense also called F.W. to testify and she stated that she was in Arizona at the time of the death. Time of death was crucial in this case and the state and the defense called entomologists to testify as to the approximate time of death as the body was decomposing at the time it was found.

The jury found the defendant guilty of the charge after a lengthy and contentious trial. He was committed to prison for life without the possibility of release as he had a prior conviction for murder on his record. The defense attorneys for the defendant were completely convinced of his innocence and encouraged the defendant to seek the services of the Innocence Project to help him seek exoneration. One piece of evidence the state never had tested prior to trial were hairs found in the hand of the victim. The Innocence Project spent considerable time and effort in seeking exoneration and also had the hairs tested. The DNA results determined that the hairs in the victim's hand were her own hair. The Innocence Project continued to comb over the evidence collected by law enforcement and determined there was no further testing that could be completed to attempt to exonerate the defendant. He has now spent more than 17 years in prison for a crime he didn't commit. It is a case the attorneys will never cease thinking about.

Innocent, Not Exonerated, Minnesota

F36 The Shifted Paradigm: The Unprecedented Year in Bitemark Analysis and Hair Microscopy Litigation

Maxwell Christopher Fabricant, JD, The Innocence Project, 40 Worth Street, Ste 701, New York, NY 10013; and Dana Delger, JD*, The Innocence Project, 40 Worth Street, Ste 701, New York, NY 10013*

The goal of this presentation is to discuss the Innocent Project's (IP's) highly publicized litigation concerning bitemark evidence and hair microscopy over the past year. More specifically, this presentation will discuss in detail two post-conviction cases, one resting entirely on bitemark comparison evidence and another on hair microscopy evidence provided by a Federal Bureau of Investigation (FBI) hair analyst. This presentation will also discuss the IP's request for an audit of all bitemark convictions and the ongoing audit of hair comparison cases, including recent state-based initiatives.

This presentation will impact the forensic science community by elucidating novel litigation strategies around newly discredited forensic techniques that have been used to secure convictions for decades. The IP is litigating cases involving such evidence on behalf of clients in California, Mississippi, Texas, Massachusetts, and North Carolina. Each jurisdiction presents different challenges and opportunities. In Texas and California, for example, there are new statutory schemes that provide avenues for *habeas corpus* relief in cases in which the forensic evidence used to convict the defendant has been discredited. This presentation will discuss how and why bitemark analysis and hair microscopy have been totally discredited and how the audits of these cases are playing out in individual cases around the country.

Over the past year, there has been a sea change in the way courts and the forensic science community must consider the admissibility and probative value of bitemark and other pattern and impression forensic disciplines. The impetus for this change has its roots in the 2009 National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward*, but has accelerated in light of continued DNA exonerations, the reexamination of thousands of convictions based on hair comparison evidence, and the IP's continued litigation against the admissibility of bitemark comparison evidence and IP's efforts to bring justice to individuals who have been wrongfully convicted through the use of these techniques.[1]

The IP anticipates significant decisions in many, if not all, of the cases noted above by the end of 2015. How these cases are decided will have an impact nationally on how courts across the country handle litigation related to audits of prior convictions resting on what is known today to be unreliable evidence. This presentation will be an opportunity to learn and discuss how this new area of post-conviction litigation will impact the forensic community.

Reference(s):

1. National Research Council, Committee on Identifying the Needs of the Forensic Sciences Community, *Strengthening Forensic Science in the United States: A Path Forward*, (2009).

Forensic Odontology/Bitemark, Hair Microscopy, Litigation

F37 The Stingray® Revolution: How the Widespread Use of Cell Site Simulators Is Changing Law Enforcement Tactics and Criminal Prosecutions in Maryland

Jason D. Ricke, JD, LLM, Office of the Public Defender, 14735 Main Street, Ste 272B, Upper Marlboro, MD 20772*

After attending this presentation, attendees will understand how the use of a cell site simulator, known informally as Stingray®, is impacting criminal trial courts in Maryland and should be a concern for the greater forensic science community.

This presentation will impact the forensic science community by illustrating how the frequency of smart phone evidence in criminal cases makes the impact of its misuse substantial. Law enforcement agencies are using Stingray® as a forensic tool, oftentimes without court authorization, and attorneys and judges alike are scrambling to decide how to deal with this emerging technology.

As of early 2015, nearly 64% of American adults own a smart phone, a number that has nearly doubled from only 35% in 2011.[1] Smart phones contain call records, messages, location information, Global Positioning System (GPS) coordinates, audio/video, photographs, financial information, fingerprints, and even facial scans. The majority of these advancements in smart phone technology have become mainstream over the past five years.

The Supreme Court most recently recognized the importance of smart phones in Riley v. California.[2] The Court stated "modern cell phones are not just another modern convenience … with all they contain and all they reveal, they hold for many Americans 'the privacies of life.'"[3]

Nearly every defendant arrested for a crime is arrested with their smart phone. The evidence contained on these phones is a veritable gold mine of information that can be used in a myriad of ways by law enforcement and defense attorneys alike.

Law enforcement initially could gather information from the phone itself or from the phone companies, but now have new methods of obtaining smart phone evidence. One of the hot button issues is law enforcement's use of cell site simulators, known by the names Stingray® or Triggerfish.[4]

The forensic community should be concerned with any new technology being used to gather evidence with little to no oversight in criminal cases. The 2009 National Academy of Sciences (NAS) Report, Strengthening Forensic Science in the United States: A Path Forward, regarding the digital and multimedia discipline noted three challenges to digital evidence: (1) a lack of certifications/qualifications for forensic examiners; (2) agencies treating the examination of digital evidence as an investigative rather than forensic tool; and, (3) wide variability in the education, experience, and training of those practicing this discipline.[5] Stingray® presents problems for all three challenges. Some of the key topics are described below.

Current State of Technology and Future Developments: The Stingray® device used by many law enforcement agencies is manufactured and sold by Harris Corporation. The details surrounding its capabilities are carefully guarded. The Federal Communications Commission (FCC) filings for information on Stingray's® capabilities only reveal heavily redacted user manuals. Originally it was thought that the Stingray® device could only obtain a phone number, device ID, and location. It is now believed that Stingray® can read content from devices as well.[6] Once content can be pulled from the air, the lines between a virtual search and a physical search are completely blurred.

Impact of Technology on Law Enforcement: Local agencies in Maryland have confirmed using Stingray® since 2007. Due to non-disclosure agreements in place for the use of Stingray®, agencies do not discuss the use of Stingray® in their investigations. Maryland presents a unique environment in the use of this forensic tool given the proximity to many federal agencies tasked with protecting this technology. The agencies have used Stingray® in a collaborative effort with local enforcement that defer responsibility when questions do arise.

Emergence of Fourth Amendment Concerns and Discovery/Disclosure Methods: Attorneys in Maryland and across the country are scrambling to find answers. State agencies and the American Civil Liberties Union (ACLU) are filing public information act requests. Attorneys are using discovery requests and motions to suppress to ascertain when Stingray® is being used. The end result is that, rather than disclose the details of this technology to the public and the attorneys, the Federal Bureau of Investigation (FBI) has preferred prosecutors simply drop cases in which a cell site simulator was involved.[7]

The rapid expansion of smart phone technology combined with the new undisclosed methods of obtaining forensic evidence is changing criminal prosecutions in Maryland and across the country. Defense attorneys and prosecutors should be equally concerned because the use of a Stingray® device without proper oversight and disclosure leaves both sides getting stung.

Reference(s):

1. http://www.pewinternet.org/2015/04/01/us-smartphone-use-in-2015/.
2. *Riley v. California*, 573 U.S. _____ (2014).
3. *Id*. At pg. 28.
4. https://www.aclu.org/map/stingray-tracking-devices-whos-got-them.
5. National Research Council, Committee on Identifying the Needs of the Forensic Sciences Community, *Strengthening Forensic Science in the United States: A Path Forward* (2009) at p. 181.

*Presenting Author

6. http://www.theguardian.com/us-news/2015/apr/10/stingray-spying-fbi-phone-dragnet-police.
7. http://arstechnica.com/tech-policy/2015/04/fbi-would-rather-prosecutors-drop-cases-than-disclose-stingray-details/.

Stingray, Jurisprudence, Digital Evidence

F38 Loss of the Fingerprint Exemption: Implications of Changes in Professional Practice

David A. Stoney, PhD, Stoney Forensic, Inc, 14101-G Willard Road, Chantilly, VA 20151; and Paul L. Stoney, MBA, 14101-G Willard Road, Chantilly, VA 20124*

After attending this presentation, attendees will better understand the nature and implications of the fundamental changes in the latent print field that have already significantly altered the standards to which existing rules of evidence apply.

This presentation will impact the forensic science community by enabling legal and forensic science practitioners to use existing processes to implement improvements that will increase the quality of latent print evidence.

The courts and forensic science community are responding to broad changes in the forensic science profession. Acceptance of evidence and standards based on historical practice is being replaced by requirements for scientific practices and a clearer distinction between fact and interpretation. These changes are particularly significant for fingerprints.

Forensic fingerprint examination has fundamentally changed. Looking broadly at practices throughout the United States, it is clear this is a period of upheaval and adjustment. For more than a century, unlike virtually all other types of physical evidence, latent print evidence had been exempt from the requirements of universally recognized good scientific practices; specifically: (1) no well-defined procedure was required; (2) no documentation of observations was required; (3) no measurements were required; and, (4) no separation of laboratory results and interpretations was required.

For latent print evidence to meet existing rules of evidence, it must be conducted in a way that acknowledges and reduces these risks. Scientific practices are an important part of reducing and controlling risks. They also allow the detection of errors and the correction of mistakes. All scientific practices require that there is a well-defined procedure, that measurements be employed, that the results of the examination are documented, and that there is a discrete, transparent, and well-justified step from these results to their interpretation. In the legal context, exempting latent print evidence from good scientific practices meant, effectively, that rules of evidence, as applied to other forensic examinations, were not applied. Latent print work was exempt from meeting the burden of proof and exempt from meaningful confrontation of the evidence. Furthermore, decision-making, by latent print examiners, included embedded personal priorities and assumptions that encroached on the trier of fact's decision making.

The changes that have removed the fingerprint exemption will be examined. Unambiguously, good latent print examination practices have dramatically changed over the past ten years. With this change, there has been a significant alteration of the standards to which existing rules of evidence apply. At the same time, the effects of the long-tolerated fingerprint exemption are still widely seen: unacceptably risky practices, conducted and interpreted by technicians, with neither the awareness nor understanding of this risk, and operating in environments lacking the oversight, documentation, and quality management that is necessary to mitigate these risks.

There has been considerable progress moving latent print evidence toward new, more scientifically driven practices and there will be additional changes as research on such aspects as human factors, error rates, documentation, and measurements continues.[1] Change is the nature of scientific progress itself — necessarily occurring as the fingerprint exemption is set aside. How quickly the changes will be incorporated into the courts is unclear. At a minimum, there will be slow, steady, inevitable change, arising from standards and guidelines developed through the Scientific Working Group on Friction Ridge Analysis, Study and Technology (SWGFAST) and through the Organization of Scientific Area Committees (OSAC). These will be implemented in forensic operations that acknowledge and embrace the universal needs for a quality management system and for the adherence to accreditation requirements. When these operations are formally accredited, there will also be an enforcement mechanism. But how and when will such changes reach the small, local jurisdiction with a few practitioners or the large operation run for many years as an effective arm of law enforcement, administered by non-scientists, and run based on law enforcement and economic and political criteria?

For changes to extend broadly to these areas, judges must recognize that fingerprints are no longer exempt — the standards applied to all types of expert evidence also apply here — and other officers of the courts must challenge latent print examination and interpretation practices, still in widespread use, that are now: (1) recognized as scientifically deficient; and, (2) bear unnecessary and unjustifiable risk.

Reference(s):

1. Stoney D.A. Emergence of Scientific Latent Print Practices. Proceedings of the American Academy of Forensic Sciences, 66th Annual Scientific Meeting, Seattle, WA. 2014.

Latent Print Evidence, Procedures, Admissibility

F39 Black Boxes and Due Process: Transparency in Expert Software Systems

Dan Krane, PhD, 3640 Colonel Glenn Highway, Dept Bio Sci, Dayton, OH 45435; and Nathaniel D. Adams, BS, Wright State University, 3640 Colonel Glenn Highway, Dayton, OH 45435*

After attending this presentation, attendees will better understand the advances being made in the use of expert software systems for the analysis of forensic evidence and the legal ramifications of insufficient evaluation of such systems.

This presentation will impact the forensic science community, the legal community, and society by describing the difficulties surrounding the design, implementation, testing, and validation of such software from a computer science perspective and how these difficulties must be accounted for when admitting results from expert software systems as evidence in criminal proceedings.

Software has generally been used to assist the analysis of forensic evidence via two main routes: data visualization (such as spectrograms or electropherograms) and statistical calculations. Both of these routes have served primarily to expedite the work performed by human experts during their evaluations of complex data sets.

Methods for conventional analysis of evidence such as breath alcohol or DNA (and even ballot counting) are widely known, take a specific set of known input values, and produce results that can be independently confirmed by other experts using generally accepted approaches. Processes performed by humans are inherently subject to review by other humans. Human experts are rightly required to testify as to the validity of their conclusions by revealing their underlying measurements, calculations, and approaches.

Advances in our understanding of complex chemical and biological processes, statistics, and computational methods have brought us to the cusp of a new era — the development of expert software systems intended to evaluate evidence that cannot be interpreted by conventional human analysis. Where forensic scientists might summarize test results as "uninterpretable" or "inconclusive," expert software systems have begun to provide very definitive conclusions. This evolution of the use of software (from improving workflow to actually interpreting evidence) has critically important implications for the criminal justice system.

When computer software rather than human experts make decisions regarding the evaluation of evidence, an effective review of these software systems is required in order to fully evaluate the performance of the system. Expert systems must, necessarily, incorporate assumptions about the operating characteristics of the tests being evaluated. The accuracy of the conclusions reached by these systems depends on the accuracy of those underlying assumptions — *and* their implementation. If independent experts cannot identify those assumptions (ideally, by examining the underlying source code), then it is very difficult to assess the reliability of the expert systems. An early proof of this point came from an in-depth, independent review of the source code used by the Alcotest 7110 MKIII-C breath alcohol analyzer software (not even an expert system). The use of this software as an unscrutinized "black box" allowed simple programming mistakes to go undetected for years.

Unlike human analysts who can sometimes struggle to explain how their approaches are objective and based on experimentally validated rules, expert systems have the distinct advantage of being based on source code that unambiguously details the exact means by which they arrive at conclusions. If an adversary objects to some portion of an expert system's approach to solving a problem, it should be possible to scrutinize the validation study, algorithm, or source code and to precisely identify the basis of the disagreement. While it may be difficult to critically review the source code of some expert systems, failure to have the opportunity to review the entire basis of an expert computer system's conclusions raises serious and legitimate concerns about due process. Lack of access in these contexts operates, in essence, as a failure to fully have the opportunity to understand or confront significant, perhaps even the most significant, evidence in a case. Expert software systems must be held to the same standards of transparency that we have come to expect of human experts.

If the source code of a black box system were disclosed, the box would be open to independent scrutiny. The main justification for maintaining black box software is the protection of intellectual property. Courts will need to decide whether the desire for secrecy (in order to protect a perceived commercial advantage) outweighs the right of defendants to fully examine the evidence against them.

Black Box, Probabilistic Genotyping, Due Process

F40 The Legal and Scientific Landscape of a Federal Analogue Prosecution Post-*McFadden*

Heather L. Harris, MFS, JD, PO Box 43626, Philadelphia, PA 19106; and T. Douglas Clifford, JD*, Law Offices of T. Douglas Clifford, LLC, 26 Benedict Avenue, Norwalk, OH 44857*

After attending this presentation, attendees will understand the questions related to the "knowledge" requirement for a controlled substance analogue offense and the majority and concurring opinions. This presentation will discuss the scientific issues in analogue prosecutions and the impact that *McFadden* may (or may not) have on future prosecutions.

This presentation will impact the forensic science community by educating scientists, attorneys, and other interested parties on the recent United States Supreme Court decision regarding the Analogue Act and the impact it may have on future federal analogue prosecutions.

This presentation will discuss the landscape for federal analogue act prosecutions after the United States Supreme Court's decision in McFadden v. United States.[1] This presentation will discuss the main issue of this case, the "knowledge" requirement for a controlled substance analogue offense, and the opinions of the 9-0 majority, including the Roberts's concurrence. This presentation will also discuss the scientific issues in analogue prosecutions and the impact that *McFadden* may (or may not) have on future prosecutions.

The issue in McFadden was ultimately one of the defendant's mens rea, or intent. The Controlled Substances Act of 1970 requires a person to *knowingly* engage in a prohibited act. The question as applied to *McFadden* was what exactly does the government have to prove a defendant knew in order to obtain a proper conviction under the Controlled Substances Act? Does a defendant have to know the exact chemical formula of the substance? Does he have to know that the substance is on the statutory list of controlled substances? Does he have to know that people will ingest the substance for the purpose of intoxication of any form?

Those questions related to the knowledge requirement become more difficult to ascertain when the Controlled Substance Analogue Enforcement Act of 1986 is considered. By definition, analogues are not controlled substances, but they can be treated as controlled substances for purposes of prosecution if determined to be substantially similar to a controlled substance in structure and effect. *McFadden's* position was that the government needed to prove that McFadden knew of this substantial similarity. This led to the question for the United States Supreme Court: what specifically does the government have to prove regarding the defendant's mens rea under the Controlled Substance Analogue Enforcement Act? This presentation will discuss this question in the context of the recent *McFadden* decision and its potential applications in future cases.

Reference(s):

1. *McFadden v. United States*, 576 U.S. ____(2015).

Controlled Substance Analogue, Mens Rea, Controlled Substance

F41 New and Better Ways to Challenge Fire Investigators in Court Using National Research Council/National Academy of Sciences (NRC/NAS) Report Initiatives

Terry-Dawn Hewitt, LLM, McKenna Hewitt, 9057 E Mississippi Avenue, #11-206, Denver, CO 80247; and Wayne J. McKenna, LLB, McKenna Hewitt, 9057 E Mississippi Avenue, #11-206, Denver, CO 80247*

After attending this presentation, attendees will have a model for building challenges against fire investigator experts in court. Conversely, experts can use this model to improve their expert testimony and prepare to survive challenges based on *Daubert*, *Frye*, their state equivalents, or during cross-examination at trial.[1,2] This model has been developed by combining the recommendations from: (1) *Strengthening Forensic Science in the United States: A Path Forward* (the 2009 NRC/NAS Report); (2) the National Commission on Forensic Sciences (NCFS); (3) the Organization of Scientific Area Committees (OSAC); and, (4) the Report of the Texas Forensic Science Commission (TFSC) in the Willingham/Willis Investigation.[3]

This presentation will impact the forensic science community by providing information to anyone involved with civil or criminal fire litigation. Moreover, attorneys, judges, and experts from any forensic science discipline can use the model presented to gain a better understanding of how to take challenges of expert testimony to the next level based on initiatives developing from the NRC/NAS Report.

The NRC/NAS Report, published in 2009, contained 13 recommendations to overhaul forensic sciences, including minimum reporting and testimony requirements, the need for accreditation and certification, and compliance with industry standards. While the NRC/NAS Report spoke largely of forensic laboratories, fire investigations was one of the forensic disciplines this Report covered; however, since fire investigations were at the periphery of the NRC/NAS Report, in many ways it was difficult to understand the "path forward" for fire investigators from this Report. This issue of how the NRC/NAS Report applies to fire investigations was addressed two years later in Texas.

In 2011, the TFSC, investigating complaints of wrongful conviction in two arson cases, endorsed the advice of the NRC/NAS Report, but placed its recommendations in the context of overhauling one forensic discipline: fire investigations. In this context, the TFSC was the leader in the path forward for strengthening fire investigations. It emphasized the need for professional certifications and adherence to standards; however, it went further, urging that counsel aggressively pursue *Daubert*/*Frye* admissibility hearings in arson cases, recommending that judges should hold such hearings in all arson cases to ensure fire science testimony is reliable and relevant.[2]

Two years later in 2013, another initiative resulted from the NRC/NAS Report. The National Commission on Forensic Science (NCFS) was chartered as a Federal Advisory Committee, to provide policy recommendations to the federal Department of Justice. While the NCFS recommendations are not finalized, they include initiatives for universal accreditation of forensic science service providers (a term defined broadly enough to include public and private organizations employing fire investigators) and the overhaul of reporting and testimony requirements for experts, presumably applying to fire investigators as well as other forensic experts.

On the heels of the creation of the NCFS, in early 2014 the National Institute of Standards and Technology (NIST) instituted the Organization of Scientific Area Committees (OSAC) to provide practice-based guidance for each forensic science discipline, including fire investigations. One of NIST-OSAC's goals is to create registries of standards and guidelines containing the key industry standards for each forensic discipline. Further, OSAC has stated one measure of its success will be that within five to ten years, these standards and guidelines will be regularly used in court in the direct and cross-examination of expert witnesses.

While there are common themes reverberating through the NRC/NAS Report, the TFSC Report, the NCFS policy recommendations, and the NIST-OSAC initiatives, these efforts are by no means unified. Notwithstanding the difficulty of predicting the long-term effects of these initiatives, it is clear that there will be changes raising the bar for expert testimony.

This presentation will connect the dots, so to speak, setting forth in a practical way how the NRC/NAS Report and the initiatives flowing from it can be used to build a credible model in civil and criminal cases to challenge (or conversely, to support) fire investigation experts in *Daubert*/*Frye* hearings, in depositions, and in trial testimony. The basic model presented in this presentation will work for other forensic disciplines by switching references to the key standards and guidelines used in fire investigations to those that are at the foundation of other disciplines. This presentation will benefit attorneys, judges, and expert witnesses.

Reference(s):

1. *Daubert v. Merrell Dow Pharmaceuticals Inc*. (1993), 509 U.S. 579.
2. *Frye v. United States*, 293 F. 1013 (App.D.C. 1923).
3. Tex. Forensic Sci. Comm'n, Final Report Willingham/Willis Investigation 8-9 (April 15, 2011), available at http://www.fsc.state.tx.us/documents/FINAL.pdf.

NRC/NAS Report, *Daubert*, NFPA & ASTM Standards

F42 Dealing With *Daubert*: The Change to and Application of a New Evidential Standard in Alcohol- and Drug-Impaired Driving Cases

Garett M. Berman, JD, Florida Traffic Safety Resource, Prosecutor Program, PO Box 32, Dania Beach, FL 33004*

After attending this presentation, attendees will understand the differences regarding the admissibility of expert testimony under the *Daubert* and *Frye* standards of evidence in Driving Under the Influence (DUI) and other alcohol- and drug-impaired-related litigation.

This presentation will impact the forensic science community by examining the recent change in evidential standards in Florida in July 2014, through a comprehensive review of cases involving challenges to expert testimony in the areas of standardized field sobriety testing, breath-alcohol testing, urine testing, and blood-alcohol testing both pre- and post-adoption of the *Daubert* standard.

Prior to July 1, 2013, Florida adhered to the *Frye* standard regarding expert testimony. The *Frye* standard requires that an expert's opinion testimony be generally accepted in the scientific community as a prerequisite to admissibility. Essentially, this allowed for an expert's "pure opinion testimony" to be admissible without any showing of the testimony to be the product of reliable scientific principles or methodologies. In adopting the *Daubert* standard, expert testimony is admissible only if it is based on sufficient facts or data, is the product of reliable principles and methods, and the expert has applied the principles and methods reliably to the facts of the case.

There is debate among courts, counsel, and legal commentators over whether the *Daubert* standard is more lenient or more strict than *Frye*. Nonetheless, since the adoption of the *Daubert* standard, defense expert testimony regarding breath-alcohol and blood-alcohol testing has significantly been curtailed in criminal impaired-driving prosecutions. No longer are defense experts allowed to testify about factors that could possibly affect breath test results, without being able to testify that these possible factors actually did occur or affect the results reported. What was once admissible under *Frye* is no longer admissible under *Daubert*.

In one of the most significant rulings, a court recently precluded an expert's proposed testimony regarding the use of a certain type of needle gauge to collect a DUI manslaughter defendant's blood sample. The expert opined that the gauge of the needle caused hemolysis of the blood sample. In a ruling that drastically affected the defense's presentation of their case, the court sustained the State's objection and precluded the expert's testimony, as well as the testimony of two other defense experts whose testimony was based in part or in whole on the other expert's testimony.

This presentation will examine several recent objections pursuant to, and attempts to meet, the *Daubert* standard and the resulting court rulings in alcohol- and drug-impaired driving prosecutions.

Daubert, **Evidence, Breath Testing**

F43 "Maybe I'm Amazed…" Maxwell Smart and Siegfried Couldn't Have Done It Better: Crime Scene Investigation in an Argentine Prosecutor's Death — Do We Really Want to Catch the Bad Guys?

Maria Susana Ciruzzi, PhD, Hospital Nacional de Pediatría Prof. Dr. Garrahan, Combate de los Pozos 1881, Buenos Aires 1045, ARGENTINA*

After attending this presentation, attendees will be able to analyze first-hand the way crime scene investigation is conducted in a high-profile political case in Argentina.

This presentation will impact the forensic science community by showing the correlation between politics, criminal law, and forensics and how the need to settle the truth is blurred by political expediency.

Late at night on January 18, 2015, Dr. Alberto Nisman, federal prosecutor in charge of the Unit for the Investigation of Terrorist Attack at the Asociacion Mutal Israelita-Argentina (AMIA), was found dead at his luxury home in Buenos Aires City, with a gunshot wound to the head. Four days before, he had filed a complaint against Argentine President, her Minister of Foreign Affairs, two other minor political partners of the ruling party, and an ex-spy from the National Intelligence Agency, accusing them of conspiring to cover up the AMIA terrorist attack. The next day (Monday, January 19), Dr. Nisman was expected to appear before Parliament to reveal the evidence regarding his accusation.

The AMIA bombing was a terrorist attack on July 18, 1994, against one of the main Jewish institutions in Argentina; 85 people were killed and more than 300 injured. This was one of the largest attacks against Jews since the Second World War. The Argentine Jewish Community is the biggest in Latin America, and fifth-largest in the world.

Dr. Nisman was the federal prosecutor in charge of the AMIA terrorist attack investigation. On October 25, 2006, the Argentine Court, on behalf Dr. Nisman's prosecution, accused the Iranian government of being the mastermind behind the attack and Hezbollah of being the perpetrator. In 2007, the International Criminal Police Organization (Interpol) issued seven arrest warrants against the Iranian suspects. In 2013, the Argentine President signed a memorandum with Iranian authorities in order to create a Truth Commission to interrogate the suspects and attempt to reach an agreement to try them in court. This memorandum was never approved by Iranian authorities and an Argentine court deemed it unconstitutional.

Dr. Nisman was under federal protection as he had received death threats from Iranian authorities. The day he was found dead, his bodyguards took more than ten hours to enter his apartment. They never reported his absence to their superior. They didn't even try to tear down the door; instead, they took a couple of hours to ask Dr. Nisman's mother to come to the apartment and open the door. Dr. Nisman's gunshot wound to the head was caused by a gun which belonged to one of his closest collaborators at the prosecutor's office. This collaborator, a computer specialist, admitted to handing over the gun to him the night before he died. The crime scene was contaminated and badly preserved: people were walking barefooted; potential evidence was handled without gloves; the gun was cleaned at the scene with toilet paper; the Secretary for Security — the head of the police officers acting as Nisman's bodyguards — had been at the scene long before the judge and the prosecutor on duty arrived at the scene, etc.

This presentation will discuss what was dealt with incorrectly at the crime scene, by who, and why.

Did Dr. Nisman commit suicide? Was Dr. Nisman killed? Was Dr. Nisman incited to suicide? Crime scene manipulation and contamination leads us to only one answer: there will be no true chance to catch the perpetrators and to know the truth.

Dr. Alberto Nisman was considered by the Argentine Jewish community as the 86th victim of the AMIA terrorist attack.

Crime Scene Investigation, Death, Contamination

F44 Battlefield Forensics: A Precursor to Counterterrorism, Peace, and Security

Abdullah Usman, LLM, MSc, Abdullah Law Chambers, Ste 13, III Floor, Sadiq Plaza, The Mall, Lahore, Punjab 54000, PAKISTAN*

After attending this presentation, attendees will have a better understanding about the link between scientific investigation and international legal entities in countering terrorism and maintaining peace and security.

In order to combat terrorism and to acquire peace and security, this presentation will impact the forensic science community by expanding the field of battlefield forensics from incident scene management to the incorporation of a mechanism for better coordination of domestic and international legal entities in the collection of evidence and the observation of violations of international humanitarian law. This presentation will provide additional research to this rapidly developing field in the specific areas of laws of war and the use of force, thus aiding the international justice system.

Battlefield forensics is a material collection process designed specifically for the troops in the field who are fighting terrorism on the front lines. Students are trained on basic skills for known and latent print lifting, DNA collection, special photography techniques, and proper documentation practices — and to do all of this quickly and efficiently.[1]

The Joint Expeditionary Forensic Facilities (JEFF) laboratories are constantly adjusting to a dynamic operational environment that ranges from providing actionable intelligence to the military commanders on the ground to the anticipated future of training and mentoring. These JEFF laboratories operate in difficult and austere environments, process samples recovered from material or associated individuals, and support a diverse cadre of military and site exploitation teams that include: special operations task forces; weapons intelligence teams; and exploitation laboratories focused on the analysis of improvised explosive devices.[2] The Chemical Weapons Convention (CWC) distinguishes chemicals which can either be used as weapons themselves or used in the manufacture of weapons.[3]

The law relating to the armed conflict lays out, *inter alia*, the study of renouncing the war-time use of explosive projectiles, prohibiting launching of projectiles and explosives from balloons, the usage of bullets which expand or flatten easily in the human body, and usage of deleterious gases or causations of asphyxiation.[4,5]

The International Court of Justice finds that the principles as to the use of force incorporated in the United Nations Charter correspond, essentially, to those found in customary international law. They therefore accept a treaty-law obligation to refrain in their international relations from the threat or use of force.[6]

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Battlefield Forensics, Counterterrorism, Peace and Security

F45 Hospital Emergency Rooms: Please Stop the Blunders and Save the Evidence

Jayne J. Batts, MD, Clinical Forensic Services, 921 Magnolia Avenue, Charlotte, NC 28203*

After attending this presentation, attendees will understand why the Emergency Room (ER) is an unrecognized crime scene and how critical, short-lived evidence which is needed by the judicial system is altered or lost during the provision of patient care.

This presentation will impact the forensic science community by encouraging members of the judicial system to network with their local law enforcement who can in turn work with emergency personnel to develop standardized protocols for evidence collection in the health care setting.

Proper prosecution of a crime begins with the collection of physical evidence performed by crime scene technicians at the initial primary crime scene. Unlike eyewitness statements, physical evidence does not lie.

According to the Federal Bureau of Investigation's (FBI's) Uniform Crime Reporting Program, in 2013 more than one million violent crimes occurred nationwide.[1] The vast majority of these victims survived their injuries and sought medical attention; when they are transported to the ER for treatment, it becomes another "primary" crime scene.

Historically, emergency personnel treat the patient's injuries without consideration of related forensic issues. Due to the lack of training, health care providers often make mistakes in regard to the detection, collection, and packaging of physical evidence which is transported with the victim to the Emergency Department (ED). Smialek noted that, due to a lack of standardized protocols for evidence collection, during the provision of patient care, critical evidence may be lost, discarded, or inadvertently washed away.[2]

During the process of removing the victim's clothing to assess their injuries, the clothing is usually tossed onto the floor and ultimately gets discarded. In cases of victims with gunshot wounds, evidence is frequently lost or altered. Improper packaging of physical evidence induces spoliation. It is common practice to cut through the bullet hole in the clothing when exposing the patient. Alteration of projectiles occur upon removal with metal hemostats and then marking the bullet for identification purposes. These actions significantly decrease the chance a firearms expert will be able to match the bullet with one from the suspect's weapon.

Invasive procedures performed by health care providers may alter wounds or cause injuries to be confused with events occurring during the resuscitation. For example, after President John F. Kennedy was shot, he had a tracheostomy (breathing) tube placed directly through a gunshot wound in the front of his neck which interfered with his postmortem examination. It is also critical to document the characteristics and evidence associated with gunshot wounds before a patient is operated on. Debriding the wound and removing tissue usually renders it impossible to determine if it was an entrance or an exit wound. Moreover, if wounds are not properly documented at the time of injury, they will heal and their appearance will change.[2] It is not uncommon for physicians to incorrectly identify entrance verses exit wounds well over half of the time.[3] Evidence collection in the ER is problematic. In one study, poor, improper, or inadequate documentation occurred in 70% of cases with the potential for criminal or civil actions.[4] In 38 of these cases, potential evidence was either not secured, not documented, or was discarded.

Clinical forensic medicine involves the application of forensic medical techniques to living patients.[5] In the ED, these techniques include the evaluation and documentation of traumatic injuries and the collection of evidentiary material for possible medicolegal use.[5] In the majority of EDs, there are no standardized protocols for evidence collection and preservation. In addition, medical providers do not have the necessary training to understand the need to incorporate these protocols into patient care in cases with forensic implications. As a result, the mistakes made may deny the justice system access to short-lived evidence of critical significance needed in subsequent criminal or civil proceedings. The lack of training in clinical forensic medicine needs to be addressed and health care providers should have standard protocols for evidence collection as well as training much like crime scene technicians receive. If this is achieved, the judicial system will have the additional evidence they may need to successfully adjudicate cases with criminal or civil implications.

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Emergency Room, Crime Scene, Evidence Collection

F46 Risk Factors in Adjudicative Incompetency: A Case Study

Lauren Traveller, DNP, 9051 Echelon Point Drive #1015, Las Vegas, NV 89149; and Joyce P. Williams, DNP, 10809 Stansfield Road, Randallstown, MD 21133*

After attending this presentation, attendees will understand various biopsychosocial influences and potential risk factors affecting competency to stand trial in conjunction with identifying future research needs pertaining to competency evaluation.

This presentation will impact the forensic science community by offering a baseline for focusing future efforts in providing the insight and evidence-based science necessary to support and guide best practices in evaluating competency-related impairments and identifying defendants at risk.

Determinations of competency to stand trial ultimately lead to significant consequences, whether to the individual or the community. As such, the legal system needs to be sure that individuals receive proper screening, referral, and evaluation regarding competency to stand trial. This vulnerable population is dependent upon the system to ensure their legal rights are observed. Similar to a disease process, incompetency to stand trial can be related to various characteristics, attributes, or exposures. Despite this, there is minimal scientific evidence identifying what pertinent risk factors are potentially correlated with adjudicative incompetency among defendants. Most literature pertaining to competency risk factors focuses on the association between mental health and competency. A major concern inherent in this is that many other variables are lacking in these analyses. Numerous medical issues are known to have effects that could potentially impact competency to stand trial but remain largely uninvestigated. Greater knowledge of associated risk factors would enhance multidisciplinary screening and evaluation practices and therefore improve early recognition of potential issues in adjudicative competency. Early identification could improve outcomes to benefit the clients and the court system by permitting such clients to more effectively and appropriately utilize specialized judicial system resources.

A case study was conducted with the objective of collecting information regarding specific characteristics that act as risk factors for adjudicative incompetency by assessing variables known and not well known to be associated with incompetency. A small sample chart audit was conducted on individual client legal cases and pertinent medical records totaling 34 defendants charged with murder; 13 who were referred to competency court were found incompetent, 11 were found competent, and the remaining 10 were not referred to competency court. Descriptive data was evaluated to identify trends among the groups and provide a baseline targeting future efforts for improvements in the science for screening and evaluation practices to determine competency to stand trial. The most significant finding was that all groups showed similar trends in risk factors. There was an abundance of mental illness, substance use disorders, biological/chemical/toxin exposure, and exposure to violence throughout all groups.

Incompetency, Defendant, Screening

F47 Due Process Necessities for Developing National Forensic Standards: Underscoring the Need to Prevent Domination of the Process by a Given Stakeholder Interest

Andrew Sulner, MSFS, JD, Forensic Document Examinations, LLC, 220 E 57th Street, Ste 200, New York, NY 10022*

After attending this presentation, attendees will have a better understanding of the minimum acceptable due process requirements imposed upon accredited Standards Development Organizations (SDOs) by the American National Standards Institute (ANSI). Attendees will also learn about the pitfalls of having the forensic standards development process dominated by a single stakeholder interest.

This presentation will impact the forensic science community by highlighting the issues that have impeded prior standards development activities in certain forensic disciplines and by illustrating the procedural safeguards that must be implemented in any future forensic standards development activities in order to produce national standards that are true consensus standards.

On July 22, 2015, the American Academy of Forensic Sciences (AAFS) announced that it had received $1.5M in funding to become an accredited Standards Development Organization (SDO), to create standards in support of the National Institute of Standards and Technology (NIST) Organization for Scientific Area Committees (OSAC), and to make those standards freely available to all. The AAFS also announced that it has contracted with The McKiel Group to assist in developing the application to ANSI to become an ANSI Standards Developer (ASD) and to generate American National Standards, with the hope that the AAFS will be able to accept proffered putative standards from the OSAC by the AAFS Annual Scientific Meeting in Las Vegas, February 22-27, 2016.

This presentation examines the methods by which American forensic standards have been developed in the recent past and discusses the critical procedural safeguards that need to be implemented in order to achieve successful development of national consensus-based forensic standards, in conformity with guiding principles for federal engagement in standards development activities and in conformity with the minimum acceptable due process requirements imposed upon accredited SDOs by the ANSI. Recent federal guidelines and ANSI's essential due process requirements will be reviewed in detail and examples of critical issues encountered in recent forensic standards development activities pursued through ASTM, an ANSI-accredited private SDO, will be discussed to illustrate the pitfalls of having the forensic standards development process dominated by a single stakeholder interest. Ongoing challenges facing government and private sector stakeholder interests with respect to future standards development activities will also be discussed. Specific recommendations will be offered to alleviate the issues that have impeded previous standards development activities in certain forensic disciplines and to maximize the likelihood that future forensic standards development activities will be able to produce true consensus standards as fairly, efficiently, and expeditiously as possible.

Forensic Standards, SDO, ANSI

F48 "De-NIST-ing": The Evidence and Science Behind the Term

Douglas R. White, MS, 100 Bureau Drive, MS 8970, Gaithersburg, Maryland 208998970; and Mary T. Laamanen, MS, NIST, 100 Bureau Drive, Gaithersburg, MD 20899*

After attending this presentation, attendees will understand the scientific process and handling of evidence which culminates in the generation of the National Institute of Standards and Technology (NIST) National Software Reference Library (NSRL) reference data set. This data set is commonly known via the term "de-NIST-ing" as applied to deduplication of case materials.

This presentation will impact the forensic science community by showing how the deduplication of case materials is a universal step in processing. A practitioner should have working knowledge of the scientific and evidentiary foundation upon which this step rests.

The NSRL collects software from various sources and incorporates file profiles computed from this software into a Reference Data Set (RDS) of information. The RDS is used to review files on a computer by matching file profiles in the RDS. This data set assists in automated deduplication involved in determining which files are important as evidence on computers or file systems that have been seized as part of an investigation. The data set can just as easily be used to target files of interest. Such uses include detection of unauthorized software installations (e.g., in corporate or web hosting environments or in intellectual property disputes) and discovery of exculpatory evidence by criminal defense teams.

The NSRL is comprised of: (1) a collection of original software, stored for evidentiary foundation; (2) a virtual collection of software, created via digital forensics tools; (3) a freely available metadata RDS for investigative use; and, (4) a standalone research environment enabling access to all NSRL data.

A rigorous, open, scientific process is followed to preserve original data and provide metadata describing various objects and states encountered on computer systems and storage. "De-NIST-ing" is a final step in that process, performed by practitioners. The "de-NIST-ing" term obscures the underlying process, and the multiple applications in which the RDS metadata may be used: deduplication, benign identification, malicious identification, etc.

This project is supported by the United States Department of Homeland Security, federal, state, and local law enforcement, and the NIST to promote efficient and effective use of computer technology in the investigation of crimes involving computers.

DeNISTing, Deduplication, Digital

ODONTOLOGY

G1 Does Multimedia Facilitate Training in Dental Hygiene Mass Fatality Preparedness?

Tara L. Newcomb, MS, Old Dominion University SODH, 4608 Hampton Boulevard, Norfolk, VA 23529*

After attending this presentation, attendees will better understand mass fatality preparedness for dental hygienists; dental hygienists have participated in mass fatality response and show promise in acts of community service and volunteerism.

This presentation will impact the forensic science community by providing an assessment of the effective mass fatality training and radiographic imaging of dental remains specific to dental hygiene. Multimedia approaches have been identified in dental publications and curriculum; however, there are no peer-reviewed publications on what type of educational methodology should be used for mass fatality training for dental hygienists.

Training in anticipation of a mass fatality incident is important for increasing the number of skilled and deployable dental professionals for recovery efforts.[1] The defined role of a dental hygienist as a mass fatality team member includes serving as a dental registrar who manages antemortem (before death) and postmortem dental records, providing surgical assistance for jaw resections, imaging postmortem dental radiographs, and performing clinical examinations of the oral cavity as part of the postmortem or record-comparison teams.[2] Extensive training is needed and recommended because practitioners with special forensics training and experience are better able to perform the tasks required for identification.[3-8] Two educational methodologies for training dental hygienists for mass fatality and radiographic imaging of dental remains are presented.

A randomized, double-blind, pretest-posttest design was used to evaluate the effectiveness of comparable educational modules for the following two groups: (1) a control group (n=19) who received low media training; and, (2) a treatment group (n=20) who received multimedia training. For the purpose of this study, multimedia was defined as use of media for teaching, which included text and graphics, audio, and video demonstrations in an integrated way that allowed for self-pacing and repetition of reading text, listening to and visually viewing materials and/or guided demonstrations. Low media was defined as using teaching presentation software with text and graphics that also allowed for self- pacing and repetition, but only in a reading and one-dimensional visual context manner. Participants were second-year baccalaureate dental hygiene students. Study instruments included a multiple-choice examination, a clinical Competency-Based Radiology Lab (CBRL) scored via a standardized rubric, and an assessment of changed interest in mass fatality as a specialty. The results were subjected to Analysis Of Variance (ANOVA) to test for statistical significance. Both of the tested educational methodologies increased participants' pretest-posttest scores and clinical CBRL scores. Interest in mass fatality training also increased significantly for all participants ($p=0.45$). There were no significant differences between the two groups with respect to pretest-posttest multiple-choice score ($p=0.6455$), interest ($p=0.9133$), or overall CBRL score ($p=0.997$).

In conclusion, this study indicated that multimedia educational methodologies and radiology lab workshops that train participants to obtain X-rays of simulated victim remains are effective approaches for mass fatality training exercises for dental hygienists. Data gained from this research may be extended to include preparedness training and exercises for dentists and other dental personnel.

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Radiology, Mass Fatality Training, Dental Hygiene

G2 Dental Maturation and Age Estimation in Children With Down Syndrome

Laura C. Farese, MD, Via Monte Bianco 2/a, Milan 20149, ITALY; Giulia Vitale, Via Valerio Laspro 10, Salerno, ITALY; Viola Bartolini, Largo Brambilla 3, Firenze, ITALY; Claudio Baldinotti, DDS, University of Firenze, Largo Brambilla 3, Firenze, Toscana 50100, ITALY; Stefano Vanin, PhD, Queensgate, Huddersfield HD1 3DH, UNITED KINGDOM; Martina Focardi, Largo Brambilla 3, Florence 50134, ITALY; and Vilma Pinchi, PhD, via Della Resistenza 14, Murlo, Siena 53016, ITALY*

After attending this presentation, attendees will be aware of the importance of estimating the correct age in Down syndromic individuals for ethical and legal reasons.

This presentation will impact the forensic science community by focusing on the difficulties in the evaluation of dental maturation of individuals with Down syndrome due to the frequent agenesis that affects these subjects.

Background: Estimating dental age based on tooth stage and maturation is relevant for the forensic identification of decedents and living subjects. Tooth data are crucial in the reconstruction of the biological profile in criminal and administrative cases, unlawful immigration, asylum seekers, and international adoption cases. Very few scientific papers study the possible influences of genetic, chromosomal, or metabolic diseases on dental development.

Down syndrome is one of the most common chromosomal syndromes, which can lead to severe growth and developmental abnormalities. Recent birth statistics in the United States document an increasing prevalence of Down syndrome, currently occurring in 11.8 per 10,000 births. Children with Down syndrome are five times more likely to manifest hypodontia, but very few studies have examined the outcome of this pathological condition on tooth development and the age estimation assessment.

The dental eruption timing in Down syndrome children is supposedly different than that of non-syndromic individuals; many authors state that the eruption of primary and permanent teeth is delayed, that the primary teeth are not always completely formed before the age of five, and that tooth eruption in females is later than in males. Previous studies also reported different conclusions, therefore, further research in this field is needed.

Goal: The present study seeks to evaluate the possible effects of Down syndrome on dental development and the estimation of dental age compared to individuals unaffected by this genetic disorder.

Methods: A representative sample set of 136 Orthopantomographs (OPGs) were taken of Down syndrome subjects who underwent radiological examination for medical reasons from 2001 to 2014 at Meyer Hospital of Florence (Italy). The records were then subjected to the following exclusion criteria and individuals matching these criteria were excluded: older than 22 years of age or younger than 7 years of age; an unclear radiogram; symmetrical agenesis; or a clinical history of dental extractions. Fifteen records were eliminated from further analysis. A total of 121 OPGs of Down syndromic patients were analyzed, including 49 males and 72 females, whose ages ranged from 7 years to 22 years. The chronological age of each individual has been calculated in days according to the date of the X-ray examination. The mean and median differences between dental age and real age were calculated. One hundred fifty children (75 male, 75 female) without clinical evidence of developmental abnormalities or a history of familial disease was considered as the control group.

All individuals of the experimental sample were divided into four age groups: 7 years-10 years, 11 years-14 years, 15 years-18 years, and 19 years-22 years. Dental maturation of all components of each group were evaluated with two different methods: Demirijan and London Atlas.

After training and calibration, all radiographs were assessed by two experienced operators; the date of birth of each subject was not known to the operators in order to avoid any bias during the estimation procedure.

All the lower left jaw teeth were considered for analysis. In case of absence of teeth from the lower left jaw due to agenesis, the corresponding lower right tooth was considered for the examination according to the Demirijan's study. The analysis of the wisdom teeth in the lower left jaw was considered only in one case: the lower right third molar was analyzed because of left–right symmetrical third molar development as reported in the literature.

The analysis of the dentition up to the second molar and up to 16 years of age was performed with the following methods: Demirjian's original seven-tooth method was used when the seven lower teeth were present (on the left or right side); and the London Atlas was used in all other cases. The London Atlas, developed by Queen Mary University of London, facilitates the age estimation using tooth development and alveolar eruption of individuals from 28 weeks *in utero* up to 22 years of age. The Gleiser and Hunt method was used to estimate the development of the wisdom teeth for subjects up to 15 years of age.

The accuracy and reproducibility of the applied methods involved the re-examination of 12 radiographs (10%), which were randomly selected from the sample set and reassessed by two operators two months after the initial assessment. Therefore, to evaluate the intra- and inter-rater agreement, statistical tests were applied. Regular permission to perform this study was previously obtained from the Regional Ethical Committee.

Conclusion: Preliminary results from this study reveal that there are many cases of dental agenesis in individuals with Down syndrome, which presents challenges when using age estimation methods based on a predetermined tooth pattern. The study suggests the London Atlas, which facilitates the age estimations by using even a single tooth, is the most suitable for subjects with Down syndrome who are affected by multiple agenesis.

Although the poor data reported in the literature regarding dental development in Down syndromic patients and the delay in dental eruption stated this is a common feature in these patients, despite the clinical evidence, this has never precisely been quantified using identification tables relating to the methods most commonly used.

The subjects included in the experimental group were only delayed 51.35% compared to non-syndromic people, with a higher female percentage. The result of this present study shows that the speed of dental maturation is influenced by the gender, and also demonstrates a higher percentage of variability of speed of dental maturation within the range of 11 years to 14 years of age.

Forensic odontologists could experience difficulty assessing the age of these subjects using common methods and overestimate the chronological age of these special patients.

Down Syndrome, Forensic Odontology, Tooth Development

G3 Selection of Analytical Techniques for Teeth According to Conservation and Conditions After Being Exposed at Different Temperatures

Nancy Vargas Becerril, PhD, National Autonomous University of Mexico, Av Universidad 3000, Coyoacán, Copilco Universidad, Mexico City 04510, MEXICO; Marco A. Alvarez-Perez, PhD, National Autonomous University of Mexico, Av Universidad 3000, Coyoacán, Copilco Universidad, Mexico City 04510, MEXICO; Lorena Valencia Caballero, PhD, Licenciatura en Ciencia Forense, Circuito de la Investigación Científica s/n, Av. Universidad 3000, Facultad de Medicina, Ciudad Universitaria, Distrito Federal 04510, MEXICO; and Ivet Gil, PhD, Unam Facultad De Medicina, Cto. De La Investigacion S.N. Cd. Universitaria, Licenciatura En Ciencia Forense, Mexico City 04510, MEXICO*

After attending this presentation, attendees will be aware of other analytical techniques to obtain information from burned teeth.

This presentation will impact the forensic science community by presenting other analytical techniques in each case, depending on the conditions and damage inflicted by the temperature, on mineralized tissues.

Forensic science is interested in the study of tissues heated at high temperatures. Several studies have reported high temperature effects, including color change, modification of mechanical properties, crystallization, porosity, and crystal size.[1] Techniques such as Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), Mass Spectrometry (MS), and Thermogravimetric Analysis (TGA) can be used to characterize physicochemical properties in mineralized tissues exposed to high temperatures; however, the mineralized tissues become brittle, which makes analysis difficult. The best strategy for analysis of mineralized teeth exposed to high temperature is still unknown.[2]

The goal of this work was to determine suitable techniques for the analysis of burned tooth samples depending on the tooth conditions and damage. Human dental organs were analyzed after exposure to temperatures ranging between 100°C and 1,200°C (intervals of 100°C) for three hours. TGA and MS analyses identified lost mass at each temperature due to chemical reactions resulting from tissue decomposition. Dentin undergoes major mass loss at temperatures ranging between 100°C and 600°C, whereas enamel undergoes major mass loss at higher temperatures. Microstructural analyses using optical microscopy and Scanning Electron Microscopy (SEM) detected physical damage on the dental organ surfaces, color changes, and high temperature-induced embrittlement. FTIR analysis identified characteristic bands of the enamel and dentin phases at each tested temperature. The dentin bands were present from 100°C to 600°C, but were absent at temperatures higher than 600°C, whereas the enamel bands are more prominent at temperatures higher than 600°C. This indicates the elimination of the organic phase and a modification of the inorganic phase through crystallization.

These results suggest that it is possible to analyze structural changes such as the loss of organic matter in teeth exposed to high temperatures under controlled conditions; however, this technique has limited application under real-world conditions due to variations in soft tissues, environmental conditions, and time.

This work is supported by UNAM-DGAPA for funding the postdoctoral scholarship and the Cátredras Program of Consejo Nacional de Ciencia y Tecnología (CONACyT).

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Teeth, Temperature, Identification

G4 Dental Age Estimation: Root Canal Widths (RCW) of Mandibular Permanent Molars at the 18-Year Threshold

Graham J. Roberts, MDS, King's College London, Dept Orthodontics, Fl 25, Guy's Tower Wing, London Bridge, London SE1 9RT, UNITED KINGDOM; Fraser McDonald, PhD, King's College London, Fl 25, Guy's Tower Wing, Great Maze Pond, London SE1 9RT, UNITED KINGDOM; and Victoria S. Lucas, PhD, King's College London Dental Institute, Dept of Orthodontics, Fl 25, Guy's Tower Wing, London SE1 9RT, UNITED KINGDOM*

After attending this presentation, attendees will be aware of the difference in approach between the Age Estimation Database of the University of Texas (UT) system based on the balance of probability and the European System (ES) of surety near the 100% level of probability using the widths of the distal root canals of the 1st, 2nd, and 3rd molars. 1,2

This presentation will impact the forensic science community by showing that RCW of mandibular permanent molars enables reliable assignment to above or below the 18-year threshold.

Introduction: The UT system uses the data array of the final stage of dental development (Stage H) to estimate the probability of a subject being below or above the 18-year threshold.[1] The system has been useful, but recent work indicates that the system is insufficiently reliable around the ages of 17 years to 19 years.[3] This was investigated by searching for Human Biological Growth Markers (HBGM) that unambiguously indicated whether or not a subject was below or above the 18-year threshold.

Materials and Methods: A total of 2,000 dental panoramic tomographs of 1,000 females and 1,000 males evenly distributed across ages 16 years to 26 years were analyzed. All assessments were performed by two investigators. The distal roots of all three permanent mandibular molars were assessed for RCW in the distomesial direction. The observations revealed there was a distomesial gradient in young subjects, with LL6d<LL7d<LL8d (RCW-1). This resulted in a gradient in which LL6d=LL7d<LL8d (RCW-2), and, finally, LL6d=LL7d=LL8d (RCW-3).

Results: The between-rater agreement for RCW gave a kappa score of 0.9707, which is a near-perfect score for reproducibility.[4] The age estimates for RCW for Stage H were censored at 21.64 years (males) and 22.78 years (females) as shown in the table below. The results showed that males at Stage H with RCW-2 and RCW-3 were more than 18 years old, whereas females at Stage H and only RCW-3 were older than 18 years.

RCW – Score	N	$\bar{X}$ (years)	SD (years)	Minimum (years)	Maximum (years)
MALES					
RCW-1	104	19.65	1.13	17.16	21.64
RCW-2	54	18.29	1.04	18.29	21.60
RCW-3	45	20.28	0.82	18.16	21.64
FEMALES					
RCW-1	119	20.37	1.39	16.33	22.77
RCW-2	93	20.69	1.37	17.34	
RCW-3	49	21.55	1.04	18.45	22.77

Discussion and Conclusion: The HBGM of RCW shows a clear distomesial gradient in young subjects, whereas young adults do *not* exhibit this gradient. The estimate of interest is the minimum age for each RCW stage. It is concluded that RCW has the potential to be used as a valid tool to estimate whether a subject is below or above the 18-year threshold.

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3. Lucas V.S., McDonald F., Roberts G.J. Dental age estimation: The 18-year threshold - A source of error explored. Proceedings of the American Academy of Forensic Sciences, 67th Annual Scientific Meeting, Orlando, FL. 2015.
4. Landis J.R., Koch G.G. The measurement of observer agreement for categorical data. *Biometrics*. 1977. 33:159-174 doi: 10.2307/2529310.

18-Year Threshold, Root Canal Widths, Reliability of Assignment

G5 Dental Age Estimation: Root Pulp Visibility (RPV) and Periodontal Ligament Visibility (PLV) at the 18-Year Threshold

Victoria S. Lucas, PhD, King's College London Dental Institute, Dept of Orthodontics, Fl 25, Guy's Tower Wing, London SE1 9RT, UNITED KINGDOM; Fraser McDonald, PhD, King's College London, Fl 25, Guy's Tower Wing, Great Maze Pond, London SE1 9RT, UNITED KINGDOM; and Graham J. Roberts, MDS, King's College London, Dept Orthodontics, Fl 25, Guy's Tower Wing, London Bridge, London SE1 9RT, UNITED KINGDOM*

After attending this presentation, attendees will establish more effective use of a single Dental Panoramic Tomography (DPT) for age assignment of individuals either below or above the 18-year threshold.

This presentation will impact the forensic science community by the use of both RPV and PLV as additional methods to improve the accuracy of age assignment at the 18-year threshold.

Introduction: A balanced DPT sample of 1,000 females and 1,000 males in the 16.00 year-25.99 year age range was used to assess the 18-year threshold using the eight-stage tooth development scheme devised by the Anglo-Canadian research team.[1] The results demonstrated that approximately 50% of females and males in the age range of 17 years-19 years may be incorrectly assessed as either less than or more than 18 years of age.[2] It is ethically and socially unacceptable for individuals to be treated as adults when they are younger than 18 years of age. Therefore, it was proposed that better analysis of a single DPT might overcome this problem.

Materials and Methods: A modification of the four-stage method described by Olze et al. was used to assess RPV and PLV for LL8 exhibiting Stage H.[3,4] The data were censored at 21.64 years (males) and 22.79 years (females). Inter-rater agreement was achieved for both RPV and PLV.

Results: The inter-examiner Kappa scores for RPV and PLV were 0.9637 and 0.9391, respectively. For males and females, the numbers of 3rd molars at Stage H used in the analysis, after censoring, were 205 and 266, respectively. The presence of 3rd molars at Stage H with RPV-4 and PLV-4 in both males and females is a strong indicator that the individual is at least 18 years old.

Root Pulp and Periodontal Ligament Visibility: LL8, Stage H, Censored Data				
	Males		Females	
	n	Min (years)	n	Min (years)
RPV-1 100% visible	9	17.87	8	16.33
RPV-2 75%-50% visible	85	17.62	138	17.34
RPV-3 25%-50% visible	93	17.16	106	17.50
RPV-4 0% visible	18	18.67	14	18.58
PLV-1 100% visible	9	17.87	8	16.33
PLV-2 50%-75% visible	85	17.62	138	17.34
PLV-3 25%-50% visible	93	17.16	106	17.50
PLV-4 0% visible	18	18.67	14	18.58

Conclusions: The use of both RPV and PLV in LL8H for males and females is an important development in age estimation for individuals with no known birth date or birth records. Using these human biological growth markers should prevent individuals younger than 18 years of age from being treated as adults.

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2. Lucas V.S., McDonald F., Roberts G.J. Dental age estimation at the 18 year threshold a road test. Proceedings of the American Academy of Forensic Sciences, 66th Annual Scientific Meeting, Seattle, WA. 2014.
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18-Year Threshold, Pulp, Periodontal

G6 A Comparison of Dental Age Estimation Using Cameriere et al. to Other Osteological Methods in a Deceased, Undocumented Border Crosser (UBC) Population

Melinda Hacker, DDS, Carrizozo Health Center, PO Box 8, Carrizozo, NM 88301; James P. Fancher, DDS, PhD, 345 Blue Lane, PO Box 682, Martindale, TX 78655; and David R. Senn, DDS, University of Texas HSC San Antonio, 7703 Floyd Curl Drive, Mail Code 7919, San Antonio, TX 78229-3900*

After attending this presentation, attendees will understand the potential role of forensic odontologists in reducing age estimation ranges reported by anthropological methods using a dental age estimation technique.

This presentation will impact the forensic science community by helping to construct a biological profile for unidentified, deceased UBCs by comparing the dental age estimation results of a non-destructive dental technique to the results of an array of anthropological age estimation techniques that have been completed for these individuals. Validating the technique against other anthropological techniques will serve to increase the tools available to the forensic investigation team.

Hundreds of migrants die every year in borderlands along the approximately 2,000 mile United States-Mexico border. Some of these UBCs in Texas have been exhumed and brought to the Forensic Anthropology Center at Texas State University (FACTS) for processing, examination, and storage until the deceased individual can be identified. Age estimation is an important step in building a biological profile to help identify unknown deceased individuals. Anthropological techniques for age estimation can be performed using complete or partial skeletal remains; however, if only skulls or mandibles are recovered, dental age estimation may be the primary identification technique utilized. This study estimated dental age for 20 UBCs at FACTS using the non-destructive dental technique described by Cameriere et al.[1]

Twenty sets of remains were chosen for study. The remains were subjected to two exclusion criteria: (1) skeletons must have at least one maxillary or mandibular canine that had naturally separated from the jaw during tissue decomposition or was easily separable; and, (2) the selected canines could not exhibit any sign of abnormal occlusion or other pathological changes. Sixteen sets of remains had both maxillary and mandibular canines meeting or exceeding the criteria, and four had only one canine available for evaluation. When possible, ipsilateral canines were chosen. Periapical dental radiographs were obtained for each canine using labiolingual and mesiodistal projections. The radiographs were then analyzed using a photo editing software. The outlines of the areas of the tooth and pulp were identified in both projection images and used to calculate tooth and pulp areas. The software facilitated the calculations by measuring pixel counts for tooth and pulp areas. The ratio between these values was used to estimate dental age according to the equations outlined by Cameriere et al.[1] These results were then compared to the results of an array of standard anthropological age estimation techniques that had been completed for these individuals. All dental age estimations performed to date have been consistent with the anthropological estimates. The method of Cameriere et al. has a standard error of ±3.62 years.[1] This method allows investigators to estimate the ages of the unknown individuals with greater precision and report narrower age-range values that could improve the likelihood of subsequent positive identification. The study is ongoing. The current results are consistent with results obtained from osteological studies but have improved precision.

Reference(s):

1. Cameriere et al., 2007, Age Estimation by Pulp/Tooth Ratio in Canines by Mesial and Vestibular Peri-Apical X-Rays. *J Forensic Sci* 2007; 52:5 1151-1155.

Dental Age Estimation, Undocumented Border Crossers, Forensic Odontology

G7 Validating Tooth Development Staging Techniques Based on the Prediction of the Mature Root Lengths

Patrick W. Thevissen, PhD, KULeuven, Dendermondsesteenweg 483, Sint-Amandsberg, Oost Vlaanderen B-9040, BELGIUM; Baraa Khalaf, MSc, KU Leuven, Kapucynenvoer 7, Leuven B3000, BELGIUM; Steffen Fieuws, PhD, Kapucijnenvoer 7, Leuven B3000, BELGIUM; and Guy Willems, PhD, Katholieke Universiteit Leuven, School of Dentistry, Kapucijnenvoer 7, Leuven B-3000, BELGIUM*

After attending this presentation, attendees will be aware of an established standard used to verify the correct classification of tooth development stages based on the prediction of the mature root length(s).

This presentation will impact the forensic science community by proving that tooth development staging techniques based on the prediction of the mature root length(s) at the moment the observed tooth is still in development has disadvantages for use in forensic age estimation investigations.

Several staging techniques were established to classify tooth development. Most of these techniques used prediction of the final root length as a reference to classify the proportionally relative stage of observed root development. For example, the technique of Köhler et al. defines the developmental stage "Root ½" as teeth with a root length equal to or longer than half of the predicted final root length and shorter than the proportional root length of the next developmental stage, which is designated as "Root ¾" and is 75% of the predicted final root length.[1] Predicting the final (mature) root length while the observed tooth is still undergoing development is a meticulous and observer-dependent (biased) task. The current study was undertaken to evaluate the validity of staging techniques based on prediction of mature root length(s).

This retrospective study collected 119 series of digital dental panoramic radiographs from the dental clinic files of the Katholieke Universiteit Leuven (KUL), Belgium, and included 63 female and 56 male subjects. Development of the second molars was evaluated for all radiographs. Each series included at least two panoramic radiographs registered from the same subject at different times. The chronologically last-registered radiograph contained mature second molars. All second molars were evaluated and staged by eight observers according to the method of Köhler et al.[1] The ratio between the second molar root lengths measured in the last-registered radiograph and in each previously registered radiograph was calculated for each subject. The degree of root length development corresponding to each Köhler stage is defined as follows: root ¼ developed, Stage 5; root ½ developed, Stage 6; root ¾ developed, Stage 7; and root fully developed, Stage 8-10. Therefore, the calculated range of second molar root length ratios that confirm correct Köhler staging are as follows: range=0.25 to <0.50, Stage 5; range=0.5 to <0.75, Stage 6; range=0.75 to <1, Stage 7; ratio=1 (i.e., no range), Stage 8-10. The calculated ratios and registered Köhler stages were independently verified for each second molar position and for each of the eight observers. Köhler staging bias was evaluated by considering that if Köhler staging was not biased, the expected mean ratio at each stage should be as follows: ratio=0.375, Stage 5; ratio=0.625, Stage 6; ratio=0.875, Stage 7; ratio=1, Stage 8-10.

Perfect differentiation between consecutive registered Köhler stages was not detected for every second molar root length and every observer. Verification of the calculated ratios and registered Köhler stages revealed that all observers generally classified the developing tooth as a more advanced stage than the correct stage, except for Stage 5. Therefore, significant observer bias was detected for all Köhler stages except for Stage 5, which did not have significant observer bias.

Longitudinal collection of subjects with panoramic radiographs containing developing second molars and fully developed, mature second molars provided exact information regarding the final root length(s) of the evaluated molars. This study developed a standard for correct tooth development staging based on prediction of final root length. The study identified significant discrepancies in observer evaluations and classifications of consecutive tooth stages. Therefore, this study proposes that staging techniques for developing teeth that are based on predictions of mature root lengths should only be performed after adequate observer training and calibration. The present study provided a useful tool that can be used for observer training and calibration.

Reference(s):

1. Köhler S., Schmelzle R.L, Püschel K. Development of wisdom teeth as a criterion of age determination. *Ann Anat.* 1994. 176: 339–345.

Dental Age Estimation, Tooth Development Staging, Tooth Maturation

G8 Combining Radiographically Observed Craniofacial and Tooth Developmental Age Predictors

Parul Khare, MSc, KU Leuven, Kapucynenvoer 7, Leuven B3000, BELGIUM; Guy Willems, PhD, Katholieke Universiteit Leuven, School of Dentistry, Kapucijnenvoer 7, Leuven B-3000, BELGIUM; Steffen Fieuws, PhD, Kapucijnenvoer 7, Leuven B3000, BELGIUM; and Patrick W. Thevissen, PhD, KU Leuven, Dendermondsesteenweg 483, Sint-Amandsberg, Oost Vlaanderen B-9040, BELGIUM*

After attending this presentation, attendees will be aware of the fact that multiple craniofacial variables registered on panoramic and cephalometric radiographs are potentially providing age-related information in children and subadults.

This presentation will impact the forensic science community by enlightening the rationale that age related information from different craniofacial variables added to dental age-related information does not contribute to improving the age estimation performances based only on dental information.

Human age estimation is an important component of protocols for the identification of unknown individuals, and it is an essential component for estimating the age of living individuals who lack age documentation so that they can obtain legal, administrative, and social rights and benefits. Dental and skeletal parameters have been combined to potentially improve age estimation performance. The current study assesses age-related craniofacial variables registered on Panoramic (PAN) and Cephalometric (CEPH) radiographs because they are easy to obtain in dental practices. Therefore, radiologically registered skeletal age-related information could be obtained during forensic age estimation analysis without the need to consult a general radiologist. The goal of this study was to identify craniofacial age-related variables registered on PAN and CEPH radiographs, and to combine the craniofacial age-related information with that based on PAN radiographs.

A total of 360 PAN and CEPH radiographs were collected, which included images taken at the same times from the same subjects with age ranges between 5 years and 23 years. The images were categorized with respect to age group (one-year intervals) and gender; ten radiographs (five PAN, five CEPH) per gender and per age group were selected for analysis. Twenty craniofacial variables were evaluated. For CEPH analysis, seven linear measures between landmarks, one differential value, and six sella turcica specifications were registered. For PAN analysis, six linear measures between landmarks were registered and the lower left mandibular permanent teeth were developmentally staged according to the method of Demirjian et al.[1] The development of all third molars present was staged according to the method of Köhler et al.[2] All radiographs were imported in Adobe® Photoshop® CS6 for digital analysis and data collection. First, an age-prediction model was developed using the approach of Fieuws et al, which was based on the 11 ordinal tooth scores (seven Demirjan scores and four Köhler scores).[3] Ten-fold cross-validation was performed to establish age predictions. The Root-Mean-Square Error (RMSE) was calculated based on these ordinal scores. Second, the continuous craniofacial measurements were evaluated to determine if they contribute information to age prediction. Separate regression models were computed for PAN and CEPH radiographs, with the difference between real ages and predicted ages (based on ordinal tooth scores) used as dependent variables. A backward selection procedure, Aikake Information Criterion (AIC), was implemented (p=0.157) for model selection. A principal component analysis was performed as an alternative method to process the large number of craniofacial measurements, and the Principal Component Scores (PCS) (the required number to explain 80% of the variability) were used as independent variables in the regression models. The RMSEs were calculated using these craniofacial measurement models.

The RMSE of age estimation based on ordinal dental scores was 1.75 years. The RMSEs of age estimation based on PCS values were 1.72 years, 1.72 years, and 1.71 years for PAN, CEPH, and PAN+CEPH radiographs, respectively. The RMSEs of age estimation based on the ordinal dental scores with the PCS values added were 1.71 years, 1.78 years, and 1.83 years for PAN, CEPH, and PAN+CEPH radiographs, respectively. The error increase after adding information reflected the effect of overfitting, and this increase was significant even for the combination of PAN+CEPH information. Therefore, the addition of craniofacial information derived from PAN and CEPH radiographs resulted in approximately similar age estimations as those obtained from using information from dental variables alone. These results indicate that the addition of craniofacial information derived from PAN and/or CEPH radiographs did not significantly improve age prediction. In summary, forensic age estimations of children and subadults do not require additional information obtained from craniofacial age predictors derived from PAN and/or CEPH radiographs when dental age predictors are accessible.

Reference(s):

1. Demirjian A., Goldstein H., Tanner J.M. A new system of dental age assessment. *Hum Biol*. 1973. 45: 211–227.
2. Köhler S., Schmelzie R., Loitz C., Püschel K. Development of wisdom teeth as a criterion of age determination. *Ann Anat*. 1994. 176: 339–345.
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Dental Age Estimation, Craniofacial, Age Indicators

G9 Third Molar Age Estimation: Appropriately Censoring Stage "H" Using the Data From Two Previously Published Studies — Blankenship et al. and Kasper et al.

Jennifer A. Moore, DMD, 9 Beverly Road, Bethpage, NY 11714*

After attending this presentation, attendees will better understand what censoring of stage "H" in dental age estimation is and why it is recommended.

This presentation will impact the forensic science community by demonstrating the significance and improved accuracy associated with the utilization of appropriately censored stage "H" in third molar age estimation cases.

Introduction: The human third molar is the last tooth to undergo morphological development and normally reaches full maturation at late adolescence/early adulthood. Mincer et al. first reported statistical data that permitted age estimation using the third molar and the empirical probability that an individual had attained the age of 18 years.[1] Many subsequent studies have been performed using these data for age estimation of ancestral populations. These studies have been utilized globally to assist authorities with cases regarding immigration and legal age within civil and criminal justice systems.

All third molar studies use a staging system to assess the degree of tooth morphological development. For each defined stage, the mean age of attainment and associated measure of variability (standard deviation) were calculated using normal distribution curves; however, the final developmental stage presents unique challenges because it involves terminal tooth maturation. By convention, previous studies arbitrarily selected the upper limit of the population data set, which resulted in over-estimation of the mean age of terminal tooth development. To accurately access the mean age of attainment of final maturation for the third molar, raw data need to be appropriately censored. Appropriate censoring is defined as the calculated elimination of raw data from individuals beyond the age from which it can be determined that complete maturation has occurred in *all* individuals within the population being studied.

This study utilized data reported by Lewis and Senn, which were recalculated from data reported by two previous studies (Kasper et al. and Blankenship et al.), and subjected these data to appropriate censoring of Stage "H" to calculate mean estimated age, standard deviation, and empirical probability that the individual had attained 18 years of age.[2-4] Each of these studies utilized the staging system developed by Demirjian et al.[5] The raw data in each study were appropriately censored by eliminating data for those individuals exceeding the true chronological age of the mean value for Stage "G" plus three standard deviations. This value was defined as the upper limit for chronological age of individuals included in the calculation of Stage "H," which effectively eliminated only 0.1% of the population who were still undergoing third molar development. This method produced a more accurate estimation of age at attainment of Stage "H." This study improved age assessment based on the developmental stage of the third molar.

Reference(s):

1. Mincer H.H., Harris E.F., Berryman H.E. The A.B.F.O. study of the third molar development and its use as an estimator of chronological age. *J Forensic Sci.* 1993. 38(2): 379-90.
2. Kasper K.A., Austin D., Kvanli A.H., Rios T.R., Senn D.R. Reliability of third molar development for age estimation in a Texas Hispanic population: A comparison study. *J Forensic Sci.* 2009. 54(3): 651-7.
3. Blankenship J.A., Mincer H.H., Anderson K.M., Woods M.A., Burton E.L. Third molar development in the estimation of chronological age in American blacks as compared with whites. *J Forensic Sci.* 2007. 52(2): 428-33.
4. Lewis J.M., Senn D.R. Dental age estimation utilizing third molar development: A review of principles, methods, and population studies used in the United States. *Forensic Sci Int.* 2010. 201(1-3): 79-83.
5. Demirjian A., Goldstein H., Tanner J.M. A new system of dental age assessment. *Hum Biol*. 1973. 45(2): 211-227.

Forensic Science, Forensic Odontology, Age Estimation

G10 Dental Age Estimation in Children With Juvenile Rheumatoid Arthritis (JRA)

Giulia Vitale, Via Valerio Laspro 10, Salerno, ITALY; Claudio Baldinotti, DDS, University of Firenze, Largo Brambilla 3, Firenze, Toscana 50100, ITALY; Viola Bartolini, Largo Brambilla 3, Firenze, ITALY; Stefano Vanin, PhD, Queensgate, Huddersfield HD1 3DH, UNITED KINGDOM; Francesco Pradella, MSc, University of Firenze, Dept of Forensic Medical Sciences, L.go Brambilla, 3, Firenze 50134, ITALY; Gian A. Norelli, sez.dep.Medicina Legale, Firenze, ITALY; and Vilma Pinchi, PhD, via Della Resistenza 14, Murlo, Siena 53016, ITALY*

After attending this presentation, attendees will better understand the influence of JRA on dental age estimation.

This presentation will impact the forensic science community by providing results from dental age estimation with two different methods.

Introduction: Methods based on calcification of permanent teeth provide reliable and accurate tools for estimating the ages of children. Dental mineralization is considered to be relatively unaffected by important diseases, nutritional status, and environmental factors that can affect growth and maturation of other biological organs (e.g., the skeleton), which are routinely used for medicolegal and forensic age estimation; however, there are few published reports that specifically focus on age estimation for children affected by genetic, chromosomal, and autoimmune diseases. Therefore, the possible influence of disease on dental maturation in children has not been completely elucidated.

Goal: This study evaluates dental maturation in children with JRA and assesses whether this disease affects dental maturation.

Materials and Methods: This retrospective study analyzed a total of 120 orthopantomographs (OPGs) of 61 patients (44 females and 17 males) that were taken for clinical purposes. A total of 30 children underwent subsequent OPG examinations after a period of years. The sample age of the total study population ranged from 3.25 years to 16.18 years (average 9.24 years). A total of 19 patients had been treated with corticosteroid therapy. Digital photos of OPGs were prepared and submitted to two forensic odontologists acquainted with dental age estimation methods and procedures. Subject age and all clinical information were masked from the operators, except for the child's gender. Two different methods were utilized for age estimation: the original Demirijan method using seven teeth (D), and The London Atlas of Tooth Development and Eruption (LA). The statistician randomly selected 15 OPGs from the general population group; these were re-evaluated by the two operators to calculate intra-observer error. A control group (50 OPGs) of Unaffected Children (UAC) with similar age distribution and gender ratio was evaluated for age estimation using the D and LA methods. The age estimations for the population with JRA were compared to those for the UAC population to evaluate possible effects of JRA on dental maturation. A possible effect of corticosteroid therapy also was evaluated.

Results: Four different age estimates were obtained for each child (two operators and two methods). The inter- and intra-operator errors were considered. The availability of multiple OPGs for some individuals allowed evaluation of variable effects of JRA at different tooth developmental phases or physical age. Preliminary results indicate that there are no significant differences in age estimates for children affected by JRA and unaffected children. Statistical analyses of these results are ongoing.

Dental Age Estimation, Rheumatoid Arthritis, Forensic Odontology

G11 An Age Estimation Procedure Based on the 3D Cone Beam Computed Tomography (CBCT) Study of the Dental Pulp Volume in Adults

Vilma Pinchi, PhD, via Della Resistenza 14, Murlo, Siena 53016, ITALY; Francesco Pradella, MSc, University of Firenze, Dept of Forensic Medical Sciences, L.go Brambilla, 3, Firenze 50134, ITALY; Claudio Baldinotti, DDS, University of Firenze, Largo Brambilla 3, Firenze, Toscana 50100, ITALY; Cosimo Nardi, MD, University of Firenze, L.go Brambilla, 3, Firenze, ITALY; Martina Focardi, Largo Brambilla 3, Florence 50134, ITALY; Giulia Vitale, Via Valerio Laspro 10, Salerno, ITALY; Gian A. Norelli, sez.dep. Medicina Legale, Firenze, ITALY; and Stefano Vanin, PhD, Queensgate, Huddersfield HD1 3DH, UNITED KINGDOM*

After attending this presentation, attendees will learn a quick method that can be helpful in odontological age estimation procedures in the living and is of striking value for its accuracy, simplicity, and usefulness.

This presentation will impact the forensic science community by providing a quick and easy odontological method, useful in the current ever-more-requested age estimation procedures in the living.

Background: The dental age of adults can be estimated by analyzing progressive physiological and degenerative phenomena affecting dental tissues. The pulp-dentin complex is a dental structure that exhibits age-dependent modifications, which result primarily in reduction of pulp chamber volume due to the continual deposition of secondary dentin. This study extends a previous pilot study and evaluates the accuracy of CBCT analysis of pulp chamber volume to estimate the age of living individuals.

Materials and Methods: Two operators randomly analyzed 277 CBCT radiographs and considered the upper left central incisor. The sample contained radiographs of 110 males and 167 females between 10 years and 80 years of age. This research was designed to simplify the dental volume measurement through a geometric approximation of different parts of the tooth. The root and the pulp were approximated as elliptical cones, and the crown was approximated as an elliptical truncated cone. The volumes were computed using Osirix® software. The ratio between the pulp volume and the hard tissue volume (*PHr*) was assumed as a variable according to the following formula: $PHr=V(P)/V(H)$. This method was validated using three extracted teeth from different individuals and comparing the dental volumes calculated by CBCT with the real volumes physically measured *in vitro*.

Results: The physical measurements revealed that the CBCT analysis consistently underestimated the real dental pulp volumes by 53%-70%; however, the error occurred for estimation of both pulp and hard tissue volumes, and it tended to be eliminated when the ratio was considered. The *PHr* was statistically significant ($p < 0.001$) as an age estimate. Gender was not significantly correlated with age; therefore, it was excluded from the linear regression formula for age estimation. The highest accuracy for age estimation was obtained for the study cohort aged between 30 years and 59 years. The age estimation error for other age-group cohorts is comparable with errors reported by other dental methods for age estimation.

Conclusion: The outcomes of this study indicate that pulp chamber volume narrowing is a reliable parameter for estimating the age of living adults, and CBCT is an easy and conservative approach that enables accurate calculation of dental tissue volumes. The proposed approach reduces the operating time compared to other techniques, which also are more complex and expensive. The results were validated by comparing the calculated volumes with the physically measured real volumes, which indicates that the experimental approach is accurate. The inter-observer agreement (Intra-Class Correlation Coefficient (ICC) 0.99) was excellent, which demonstrates that the method is highly reproducible.

Forensic Odontology, Age Estimation, Cone Beam Computed Tomography

G12 A Biometric Identification System and Border Control: A Proposal for the Integration of Digital Orthopantomograms (OPGs) and Odontogram Data of Migrants

Emilio Nuzzolese, PhD, Ambulatorio Nuzzolese, Viale JF Kennedy 77, Bari 70124, ITALY; Sakher J. AlQahtani, PhD, DDS*, Institute of Dentistry, 4 New Road, London E1 2AT, UNITED KINGDOM; and Joe Adserias, DDS, PhD*, C/ Balmes 62, Barcelona, SPAIN*

After attending this presentation, attendees will better understand the standards and procedures applied when legal/illegal migrants cross borders in Italy, Spain, and Saudi Arabia.

This presentation will impact the forensic science community by presenting the results of the possible integration of dental evaluation and panoramic X-ray in the border control system for asylum seekers for the identification of illegal migrants and for age estimation.

The increase in migration across countries has led to a range of problems in public health, border control, and human rights. An investigation into border controls for asylum seekers was performed in Italy, Spain, and Saudi Arabia to evaluate the procedures in place for identification, age assessment, and general health of migrants. A result of this investigation was the proposal that dental and odontological examinations should be included in the checks performed at national borders.

For non-European Union (EU) nationals entering Europe and Saudi Arabia, entrance is controlled by passports and fingerprints. In Spain and Italy, fingerprints are taken when there are doubts about a person's identity. Those without identification documents follow a different legal path because of the need for identification and the implications for public health. Migrants are taken to a hosting center where they are lodged until they are identified. The migrants receive humanitarian assistance and health care during their stay at the hosting center, including general health evaluations, medical examinations, X-rays, blood chemistry tests, and microbiological analysis. In Saudi Arabia, all visitors must have their fingerprints taken when applying for a visa and they are checked at border control. Saudi Arabia recently introduced a campaign to fight illegal immigration (Violators Of Residence And Work System (VORAWS)) through collaboration between the Ministry of the Interior, the Ministry of Foreign Affairs, the Ministry of Labor, the Ministry of Social Services, the Ministry of Health, and the Border Agency. Migrants are held in a center while the process of extradition and/or identification takes place. Similar health controls as those described above are performed to evaluate migrant health status.

Age estimation of unaccompanied minors arriving at the borders of Spain, Italy, and Saudi Arabia is not performed using a standardized protocol. Age estimation procedures can include a physical examination and interview, wrist X-ray, panoramic OPGs, and a Computed Tomography (CT) scan of the proximal clavicle, but only at the discretion of the medical examiner. Dental age estimation methods are not always included in the age assessment protocols and judicial procedures. The preliminary results of this study indicated that dentistry and odontology are not included in the general health evaluation and forensic assessment of migrants, although dental care often seems to be requested by migrants (depending on their length of stay at the hosting centers).

The analyses of this study suggest that dentistry and dental radiology should be performed as part of the general health evaluation of migrants, and the dental fitness scores suggested by the North Atlantic Treaty Organization (NATO) and the International Criminal Police Organization (INTERPOL) should be applied. This also could help substantiate relevant identification data through the development of a database of odontographs and digital OPGs. Migrant dental records also could help identify those attempting to enter using an alias or with false, incomplete, or altered fingerprints. Dental data could additionally be used for a complete age estimation procedure for unaccompanied minors. Emphasis should be placed on the inclusion of dentistry and dental radiology in the general health evaluation of migrants. This proposal would lead to an archive of dental data that would allow better medical/dental care and provide forensic opportunities for positive identifications and age assessments.

Migrants, Age Estimation, Human Identification

G13 Utilizing Custom Spreadsheets for Age Estimation Cases

Derek M. Draft, DDS, 3100 Wilson Avenue SW, #3, Grandville, MI 49418*

After attending this presentation, attendees will possess a basic knowledge of how to utilize Excel® spreadsheets to aid forensic odontologists in the calculation of estimated age for their cases.

This presentation will impact the forensic science community by teaching the forensic odontologist the basic principles of a spreadsheet, which will allow him/her to precisely and accurately calculate the estimated age of an unknown individual utilizing established data and methods from prior published studies.

Forensic odontologists are often enlisted by agencies to estimate the age of living and deceased individuals. Forensic odontologists can calculate age estimations by utilizing published age estimation protocols and typically apply a mathematical formula and/or reference data table. The formulas and data can be easily incorporated into custom spreadsheets. A spreadsheet can be built for each age estimation protocol and each case under investigation. For example, a spreadsheet can be built utilizing data and an appropriate protocol for age estimation of adults, and separate spreadsheets can be built utilizing data and appropriate protocols for age estimation of adolescents or children.

This presentation will teach attendees some common spreadsheet formulas that can be utilized to build a custom spreadsheet. Custom spreadsheets will be presented to educate attendees on how to utilize published formulas so attendees can create their own spreadsheets. Proper methods to input measured variables will be reviewed. The customized spreadsheet then utilizes the formulas, variables, and input data to compute the estimated age, associated standard deviation, and age range. The main goal of utilizing custom spreadsheets is to increase calculation speed and accuracy for the forensic odontologist and reduce the repetitive need to access and reference the original study. Spreadsheets reduce common mathematical errors and increase the accuracy of age estimation calculations. The results of the spreadsheet calculations can be utilized in forensic odontology reports by importing the appropriate fields into the report.

In conclusion, this presentation will review and provide examples of commonly published formulas, variables, and data that can be utilized to create custom spreadsheets. Attendees will receive instructions on how to access specific variables and input data within the spreadsheet. Copies of pre-built custom spreadsheets will be provided via email to all interested forensic odontologists.

Spreadsheet, Age Estimation, Database

G14 The Performance of Willem's Method in Estimating Dental Age in Children: A Systematic Review and Meta-Analysis

Mohd Yusmiaidil P. Mohd Yusof, MS, Ghent University, PAECOMEDIS Research Cluster, UZ Ghent, Gent 9000, BELGIUM; Ilham Wan Mokhtar, MSc, Ghent University, Dept of Paediatric Dentistry and Special Care, UZ Ghent, Ghent 9000, BELGIUM; Sivaprakash Rajasekharan, MSc, Ghent University, PAECOMEDIS Research Cluster, UZ Ghent, Ghent 9000, BELGIUM; Rosanna Overholser, PhD, Ghent University, Dept of Applied Mathematics and Statistics, Sterre Campus, Ghent 9000, BELGIUM; and Luc Martens, PhD, De Pintelaan 185 P8, Gent 9000, BELGIUM*

WITHDRAWN

G15 Accuracy of the Third Molar Index for Assessing the Legal Majority of 18 Years of Age in the Turkish Population

Roberto Cameriere, Via Don Minzoni 9, Macerata, ITALY; Stefano De Luca, PhD, University of Arica, Dept of Anthropology, Arica, CHILE; Ayse Gulsahi, PhD, Baskent University, Faculty of Dentistry, Dept of Dentomaxillofacial Radiology, Ankara, Türkiye 06490, TURKEY; Burcak Cehreli, PhD, Baskent University Faculty of Dentistry, 11. sok No:26 Bahcelievler, Ankara 06490, TURKEY; and Ebru Tirali, PhD, Baskent University Faculty of Dentistry, Dept of Pediatric Dentistry, Ankara, TURKEY*

After attending this presentation, attendees will better understand age assessment based on the apical formation of third molar teeth as well as the third molar index as an indicator for legal majority. Attendees will be aware of age assessment by using teeth, jaws, and Orthopantomographs (OPGs) as alternative techniques in forensic science.

This presentation will impact the forensic science community by validation of a simple method for assessing adult age in the Turkish population with a specificity value of 100%. The method was introduced in 2008 and tested on various populations.

The age of majority is the age at which the law considers a person to have reached adulthood and attained full legal citizenship and whose decisions no longer require the oversight of a parent or guardian.[1] The Turkish Civil Code considers a person to be an adult at the age of 18 years; therefore, individuals aged ≥18 years are judged according to general criminal laws.[2] In this context, it is necessary to use non-invasive methods with high accuracy and precision for age estimation because of specific legal requirements. An important type of error in age assessment is the false adult; this is the worst and least desirable error and can have critical and unacceptable legal consequences. In 2008, Cameriere et al. developed a new method for assessing adult age based on the ratio between age and normalized measurements of the open apices and the length of the third molar (I_{3M}).[3] A cut-off value of I_{3M}=0.08 was determined to assign an individual to either a juvenile or adult age classification.[4]

The goal of this cross-sectional study was to test the accuracy of the cut-off value of 0.08 for the third molar index (I_{3M}) protocol to assess legal adult age determination in a sample of Turkish children and young adults. Digital OPGs of 293 healthy Turkish children and young adults (165 females and 128 males) between 14 years and 22 years of age were analyzed. Concordance correlation coefficient (ρ_c) and Cohen's kappa coefficient (κ) statistics indicated that repeatability and reproducibility are high for both intra- and inter-rater error. The analysis resulted in sensitivity of 85.9% and specificity of 100% for females, and sensitivity of 94.6% and specificity of 100% for males. The calculated positive and negative predictive values and the likelihood ratios for both females and males also verified the accuracy of the Cameriere cut-off of 0.08.

In conclusion, the specificity values of 100% for both females and males showed the absence of false negatives and the correct classification of all minors; however, the third molar cut-off method should be applied carefully, and it is recommended that a combination of several methods be performed to determine whether an individual should be considered as a legal adult (≥18 years) or a juvenile.

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2. Cipriani D. *Children's rights and the minimum age of criminal responsibility: A global perspective*. Ashgate Publisher, Farnham, Surrey, England/Burlington, VT, 2009.
3. Cameriere R., Ferrante L., De Angelis D., Scarpino F., Galli F. The comparison between measurement of open apices of third molars and Demirjian stages to test chronological age of over 18-year-olds in living subjects. *Int J Legal Med* 2008; 22:493–497.
4. De Luca S., Biagi R., Begnoni G., Farronato G., Cingolani M., Merelli V., et al. Accuracy of Cameriere's cut-off value for third molar in assessing 18 years of age. *Forensic Sci Int*. 2014. 235: 102.e1–102.e6.

Third Molar Index, Legal Majority, Turkish Population

G16 Standards of Dental Developmental Stages

Sakher J. AlQahtani, PhD, DDS, Institute of Dentistry, 4 New Road, London E1 2AT, UNITED KINGDOM; and Mary A. Cimrmancic, DDS, Marquette University School of Dentistry, PO Box 1881, Milwaukee, WI 53201-1881*

After attending this presentation, attendees will: (1) survey currently defined tooth development staging criteria and identify similarities and differences as a first step in defining standardized age estimation staging metrics; (2) determine whether existing staging scheme definitions are adequately defined; and, (3) propose enhanced definitions of existing stages to facilitate inter-observer reliability.

This presentation will impact the forensic science community by setting the foundation for unambiguously defining the stage of dental development leading to a standardization of methodology to aid in intra-operator and inter-operator calibration.

Chronological age is a major criterion that determines an individual's eligibility for rights, privileges, and legal responsibilities throughout life. These determinations are complicated when an individual lacks birth documentation. For children, adolescents, and young adults, estimation of their chronological age based on dental, skeletal, somatic, and psychological development can provide adjunct data for decision-making in forensic cases. For living individuals seeking asylum, undergoing adoptions, or progressing through the legal system, the probability of having reached the age of majority is an important criterion for decision-makers. For unidentified decedents, age-at-death estimates can be crucial in the formation of a descriptive biological profile, can provide data to narrow antemortem record searches, and can provide adjunct data for identification.

Several methods are used for age estimation in subadults based on tooth formation. The methods utilize different staging schemes to document the development of crowns and roots of primary and permanent teeth, resorption of primary tooth roots, and eruption of teeth. The methods used in forensic cases involving children, adolescents, and young adults require staging of dentition formation. The number of stages and their definitions vary according to the method. Some staging schemes are described in great detail, whereas others are somewhat ambiguous and subject to reader interpretation. Agreement among observers and reproducibility are essential to verify method reliability. Variations in staging schemes can be challenging for forensic personnel who must be familiar with multiple methods in order to provide accurate age estimations. These variations pose even greater challenges for training new forensic odontologists and technicians.

Tooth Development, Standards, Dental Age

G17 Third Molar Maturity Index (I_{3M}) for Assessing Age of Majority in a Black African Population in Botswana

Jelena Cavric, DDS, Ivana Lucica 3., Gunduliceva 5, Zagreb 10000, CROATIA; Marin Vodanovic, PhD, Gunduliceva 5, Zagreb, CROATIA; Serena Viva, PhD, Age Estimation Project, University of Macerata, Macerata, ITALY; Laura Paula Reu, PhD, Age Estimation Project, University of Macerata, Macerata, ITALY; Roberto Cameriere, Via Don Minzoni 9, Macerata, ITALY; and Ivan Galic, School of Medicine, Šoltanska 2, Split 21000, CROATIA*

After attending this presentation, attendees will better understand that I_{3M} may, with high accuracy, discriminate individuals of Black African origin who are approximately the legal adult age of 18 years in Botswana.

This presentation will impact the forensic science community by illustrating that I_{3M} may be a useful alternative method in legal and forensic practice.

Assessment of legal age, also known as the age of majority, is a controversial issue because of a lack of biomarkers or physiological evidence during late adolescence that can be used to differentiate a subject as a minor or adult. The third molar has been recognized as a suitable site for age examination in late adolescence.

This study analyzed the development of the left mandibular third molar using I_{3M} and a specific cut-off value of I_{3M}=0.08, which was established by Cameriere et al. and was used to discriminate between minors and adults.[1] A final sample of panoramic radiographs (Orthopantomograms (OPTs)) of 1,294 living Black Africans from Gaborone, Botswana, obtained from 582 males and 712 females aged between 13 years and 23 years, was evaluated. The real chronological age declined as I_{3M} gradually increased. There was no statistically significant difference in third molar development between males and females (p >0.05) across different I_{3M} classes. Results of a 2×2 contingency table indicated that I_{3M}=0.08 was a useful cut-off value for discriminating between minors and adults. The accuracy or overall fraction of correctly classified participants was 0.91 with a 95% Confidence Interval (CI) of 0.89-0.93. The test sensitivity, or the proportion of participants assessed as 18 years and older, was 0.88 (95% CI 0.87-0.89), whereas test specificity, or the proportion of individuals assessed as younger than 18 years (I_{3M} <0.08) was 0.95 (95% CI 0.93-0.96). The positive predictive value of the test, or the prediction that the participants whose I_{3M} <0.08 were adults, was 0.95 (95% CI 0.94-0.97). The negative predictive value of the test, or the prediction that the participants whose I_{3M} was ≥0.08 were minors, was 0.88 (95% CI 0.86-0.89). The Likelihood Ratio of the positive test (LR+) was 17.69 (95% CI 12.91-24.79). The Likelihood Ratio of the negative test (LR-) was 0.12 (95% CI 0.11-0.14). The estimated post-test probability p was 0.95 (95% CI 0.92-0.97).

These results indicate with high accuracy that calculating I_{3M} may be a useful alternative method in legal and forensic practice to discriminate individuals who are approximately the legal adult age of 18 years in a Black African population in Botswana. Further studies should address the usefulness of this method and specific cut-off values for different adolescent populations.

Reference(s):

1. Cameriere R., Ferrante L., De Angelis D., et al. The comparison between measurement of open apices of third molars and Demirjian stages to test chronological age of over 18 year olds in living subjects. *Int J Legal Med*. 2008;122(6):493-497.

Botswana, Third Molar Maturity Index, Age of Majority

G18 Domestic Predation of an Elder: A Fatal Dog Attack Case

Erwan Le Garff, MD, Institut Médico-légal/Forensic Institute, Rue André Verhaeghe, Lille Cedex, Nord 59037, FRANCE; Yann Delannoy, MD, Forensic Taphonomy Unit, Rue André Verhaeghe, Lille 59000, FRANCE; Vadim Mesli, MD, Institut Medico Legal, Rue Andre Verhaeghe, CHRU Lille, Lille Cedex, Nord 59037, FRANCE; Valéry C. Hedouin, MD, PhD, Iml-chu Lille, Rue Andre Verraeghe, Lille 59000, FRANCE; Anne A. Becart, DDS, PhD, Service De Medecine Legale, Rue Andre Vaerraeghe, Lille 59000, FRANCE; and Didier Gosset, MD, PhD, Institut de Medecine Legale, Faculte de Medecine, Lille 59045, FRANCE*

After attending this presentation, attendees will be familiar with a case of a fatal dog attack with typical skin and bone bitemarks and the injury mechanism that occurs in such cases.

This presentation will impact the forensic science community by providing an example of a lethal dog attack, which happened to an elderly person at home, with examples of the risk factors of a fatal dog attack, a previous domestic predation, and the past medical history of the victim. Furthermore, this presentation explains the legal process that occurs in France in similar cases.

Domestic animals can cause severe injuries to bone and skin during an attack. Domestic attacks are relatively common, but few of them are lethal. Presented is the case of a 91-year-old woman with Alzheimer's disease who was found dead at her home by her daughter. The decedent's body was found near her wheelchair in a supine position on the floor in a pool of blood. The woman lived alone with two domestic dogs, a Labrador Retriever and a Staffordshire Bull Terrier. Shortly after the discovery of the decedent, police investigations discovered blood stains on the jawbone of the Staffordshire Bull Terrier. Food supplies were present in the home. The Staffordshire Bull Terrier had previously attacked the decedent two years prior, inflicting a right ear laceration and a right ocular wound. External examination, autopsy, and radiographies detected multiple skin and bone lacerations of the scalp, face, left arm, and limbs. There were complex bone fractures on the left humeral and right face. Body cavities were intact and the airway was free of blood, but exsanguination and discrete general atheroma were found. No scavenging marks were observed. The punctures and tearing of each skin wound were compatible with bitemarks. Histological analysis showed that skin wounds and bone fractures were vital lesions. Similar distances were found between the Staffordshire Bull Terrier teeth and the punctures and tooth marks of the major bitemarks. Death was attributed to an external hemorrhage due to several dog bites to the face and limbs.

Domestic animal attacks can cause slight to severe injuries, but death is rare. Epidemiological studies regarding domestic dog predation indicate that children and elderly women are most often targeted. Dog attacks are generally directed toward limbs in order to immobilize, and the incapacitation is due to neck or head injuries with unique or associated asphyxiation, exsanguination, or skull trauma. The victim was unable to move due to advanced neurological disease and could not protect herself during the attack or contact someone afterward. The cause for the dog's behavior was questioned. In the present case, food was readily available and the injuries occurred when the victim was alive. These conditions do not support the "hunger hypothesis" proposed in previous studies. A "reviving hypothesis" proposes that the dog's licks and bites are performed to stimulate the dog's unconscious owner. This is not the main hypothesis in the current case because the victim was alive during injury, and there were multiple injuries with spaced distribution of the lacerations; however, this hypothesis cannot be ruled out. A "domestic predation" hypothesis is preferred in the present case due to this dog's previous history of attack and because a "dangerous breed" such as Rottweiler or Staffordshire Bull Terrier is often involved in domestic attacks compared to other dog breeds. In France, dangerous dogs have to be reported to the authorities and the owners have to be certified to possess these dog breeds. In the case of a mild attack, the dog has to be monitored by a veterinarian for rabies. In the case of a vicious attack with severe injuries, the authorities can euthanize the dog. Despite a previous attack on the same person two years before the fatal attack, the dog was not evicted from the home.

This presentation highlights the case of an indoor domestic dog predation on a vulnerable elderly woman. The cohabitation of a potentially dangerous animal and a weakened person may have dramatic consequences, especially when the dog has a previous history of aggression to the victim.

Animal, Bite, Forensic

G19 Dental Cone Beam vs. Microfocus Computed Tomography (CT) in Dental Pulp Volume Calculation for Estimating Age in Adults for Forensic Purposes

Claudio Baldinotti, DDS, University of Firenze, Largo Brambilla 3, Firenze, Toscana 50100, ITALY; Vilma Pinchi, PhD, via Della Resistenza 14, Murlo, Siena 53016, ITALY; Lucia Mancini, PhD, Sincrotrone Trieste SCPA, Area Science Park, Basovizza, Trieste 34149, ITALY; Francesco Pradella, MSc, University of Firenze, Dept of Forensic Medical Sciences, L.go Brambilla, 3, Firenze 50134, ITALY; and Giulia Vitale, Via Valerio Laspro 10, Salerno, ITALY*

After attending this presentation, attendees will understand how to evaluate the accuracy in the calculation of dental volumes (pulp and hard tissues) when using a microfocus-CT (μ-CT) scanner and a software for the calculation of the reconstructed volumes.

This presentation will impact the forensic science community by showing methods based on Cone Beam Computed Tomography (CBCT). This could be the first choice for estimating age in corpses or ancient teeth.

Background: Dental pulp shows age-related changes, which primarily reduce the pulp chamber volume due to the continual deposition of secondary dentin. The recent development of technology for 3D radiological imaging of teeth, such as the modern dental CBCT and μ-CT, enables a non-destructive approach for dental age estimation based on the correlation of pulp reduction with aging of human adults. CBCT uses low-dose X-rays (similar to that used for Orthopantomographs (OPGs)) for 3D imaging, and can be used in living subjects for forensic purposes if the Field Of View (FOV) is small. Conversely, μ-CT uses high-dose X-rays for imaging and can be performed only for postmortem analysis.

In a previous pilot study, it was verified that a geometric approximation based on CBCT images enabled calculation of dental volumes (pulp and hard tissues) within ten minutes using standard hardware. The calculated ratio between pulp volume and hard tissue volume (*PHr*) correlated with the age of the subject. In the same study, the proposed geometric approximation by an *in vitro* physical measurement of dental volumes in three extracted central incisors was validated. This analysis indicated that dental volumes calculated via CBCT regularly underestimate the real volumes by 53% to 67%. Because this error occurs quite regularly for both pulp and hard tissue volumes, it tends to be eliminated when considering the ratio (*PHr*). Therefore, the age estimation results are statistically accurate, especially for cohorts aged 30 years-59 years.

Goal: This study evaluates the accuracy of computed dental volumes (pulp and hard tissues) when using a μ-CT scanner and software for calculating the reconstructed volumes. This method will be compared to the CBCT method and geometric approximation. If μ-CT outperforms CBCT methods, it could be the first choice for postmortem age estimation.

Materials and Methods: The same central upper incisors used for the validation procedure in the prior study were scanned by μ-CT at the TomoLab station of the Elettra-Sincrotrone Trieste laboratory in Basovizza in Trieste, Italy. The commercial software COBRA was used for 3D reconstruction of the sample volumes, and the Pore3D software (developed and customized at Elettra) was used for quantitative analysis of the volumes. The ratio between pulp and hard tissue volumes (*PHr*) was assumed as a variable according to the following formula: $PHr = V_{pulp}/V_{ht}$. The *PHr* ratios obtained from μ-CT and CBCT/geometric approximation analyses were compared, then these results were compared to the *in vitro* measurement of real dental volumes performed by the Physics Department at the University of Naples.

Discussion: The volumes obtained by μ-CT, CBCT, and physical examination will be discussed. The accuracy and suitability of the two approaches for different cases will also be evaluated.

Age Estimation, Computed Tomography, Forensic Odontology

G20 Development of Preliminary Field Morgue Hazardous Materials (HAZMAT) Entry/Triage Flow Protocols Initiated on a Coordinated Field Training Exercise (FTX) Between the Suffolk County, New York, Medical Examiner's Office and the Disaster Mortuary Operational Response Team (DMORT) 2 (10/2/14, Revisited)

Richard Boguslaw, DMD, 206-07 Hillside Avenue, Hollis Hills, NY 11427*

After attending this presentation, attendees will be aware of the early design parameters and problems which arose and were addressed when initiating a non-radiological/nuclear (non-rad/nuc) field morgue entry/triage and HAZMAT area and flow on a coordinated multi-agency field training exercise.

This presentation will impact the forensic science community by emphasizing considerations which need to be addressed when initiating a basic flow design for a non-rad/nuc HAZMAT field morgue entry and triage operation in a mass fatality event.

The proper handling of hazardous materials early during the recovery of remains and morgue processing is crucial for optimum safety and protection. This has to be implemented at every step down the line to the field morgue. The intake, triage, and HAZMAT decontamination (decon) of remains must predictably provide safety and protection, from the earliest stages of recovery to the reception and intake of remains.

On September 28, 2014, the Suffolk County Medical Examiner's (SCME) Office participated with Islip Airport Fire Rescue in their annual airport drill conducted on the grounds of Islip Airport (MacArthur Field, NY). The scenario simulated a crash on takeoff of a commercial jet with aviation fuel contaminating the remains. Fuselage fragmentation was reported, and the presence of severed bodies, some commingled Human Remains (HR) on the plane and field, and burnt remains were reported. Emergency plans would have identified the Army Aviation Support Unit's Facility as the medical examiner's operation area. In conducting this drill, SCME worked closely with the Army and utilized their hanger adjacent to the airport property. Components of this drill occurred at the actual crash site and the field morgue. The multi-agency response was coordinated with the county's HAZMAT Unit, which was composed of civilian, law enforcement, and fire-service members. The HAZMAT Unit was used to decon the mannequins as they were delivered to the hanger's temporary morgue. In addition, drill participants had to be prepared for possible biological and Improvised Explosive Device (IED) threats. The most important drill objectives were the safety and accountability of participating members. The drill objectives included the following: (1) review of Incident Command System (ICS) forms and protocols; (2) participants follow the chain of command, accountability, and safety rules; (3) effective coordination and interaction between federal (DMORT), state (Army Aviation Unit), and local SCME and Fatality Response System (FRES) participants; (4) documentation and recovery of five field mannequins; (5) admission, triage, and numbering of at least ten mannequins; (6) initial HAZMAT Team decon of at least three mannequins as they were delivered from the field to the temporary morgue operation; (7) appropriate Personal Protective Equipment (PPE) was utilized at each station; (8) electronic collection and transmission of data; and, (9) establishing clear lines of communication between FRES and ME/DMORT staff.

The following work assignments were appointed: (1) establish a triage area adjacent to the HAZMAT area; (2) examine HR pouches that arrive from the crash scene before they enter decon; (3) ensure each pouch contains only one individual HR — separate the comingled remains as needed; (4) assign a unique and individual ME number for each HR before decon; (5) function as an Subject Matter Expert (SME) for HAZMAT teams; (6) admission and triage table positioned at the entrance to the hanger morgue area; (7) triage will receive pre-packaged HR pouches; (8) pouches will be opened, photographed, and the contents will be documented with special emphasis on assigning an individual ME number to HR as identified and potentially identifiable; (9) separate non-human remains to be transported to morgue; and, (10) ME number will be issued by team documentation unit.

The drill, as in most staged events, started with initial delay and confusion; however, early flexibility and smoothly shifting the original Incident Command System (ICS) assignments as required resulted in a successful exercise. All personnel acted as a seamless, effective unit. The initial flow schematic developed from the basic (rough) outlay to a more detailed outlay as the day progressed. An after-action submission of the final flow-chart design is presented as a model developed from the drill results that may be adopted for future drills. This has been submitted to the local SCME Office. At the conclusion of the event, the four groups assembled in a work area and established a workable action plan to enter, triage, number, decon, and process HR while maintaining a strict chain of custody and command.

HAZMAT, Triage, Flow

G21 Bitemarks — Maybe It Is Rocket Science

Ken F. Cohrn, DDS, Forensic Consultant, 422 Teague Trail, Lady Lake, FL 32159*

The goal of this presentation is to promulgate the merits of bitemark evidence in forensic science.

This presentation will impact the forensic science community by providing a forum for bitemark discussion.

Forensic identification sciences, in particular bitemarks, have been the bane of forensic science since the publication of the 2009 National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward*. Disdained by some in legal and academic communities and accepted by others as viable forensic evidence, the questions of "admissible or not?" and "science or junk?" have arisen.

The 2009 NAS Report delivered a controversial criticism of perceived and/or real shortcomings of much of the forensic sciences. In particular, there were two questions regarding the admissibility of forensic evidence in criminal trails. First, what is "the extent to which a particular forensic discipline is founded on a reliable scientific methodology that gives it the capacity to accurately analyze evidence and report findings."? Second, what is "the extent to which practitioners in a particular forensic discipline rely on human interpretation that could be tainted by error, the threat of bias, or the absence of sound operational procedures and robust performance standards."? Is forensic identification science, including bitemark evidence, "normal" science with approaches and techniques like DNA typing with its measureable attributes, sampling of variation in populations, and statistical basis?

Bitemark scrutiny has occurred on many fronts, including "experts" from the media and blogs, errors in judgment and opinions, investigative errors, lack of research, conflicting expert testimony, bias, and misconduct. Basic issues inherent in bitemark analysis and interpretation include: (1) the uniqueness of human dentition has not been scientifically established; (2) the ability of dentition to transfer a unique pattern to human skin; (3) the ability of the skin to maintain that unique pattern; and, (4) the interpretive process is experientially based rather than scientifically based. This rancor exists in the legal community and among forensic odontologists themselves. Some odontologists have opted out of the bitemark business altogether and have even recanted on the validity of the value of bitemark analysis.

Despite the controversy, there are still many in the investigative, legal, judicial, and professional disciplines that argue that bitemark analysis remains a viable tool in the forensic process. This presentation will offer opinions to support that conclusion.

Bitemarks, Evidence, Validity

G22 Scorched Earth Forensics — Why The Move to "Eradicate" Disciplines From the Courtroom Is Bad for Science and Bad for the Law

Melissa Mourges, JD, New York County District Attorney's Office, One Hogan Place, New York, NY 10013; and Roger D. Metcalf, JD*, Tarrant County, Medical Examiner's District, 200 Feliks Gwozdz Place, Fort Worth, TX 76104*

After attending this presentation, attendees will explore the debate behind calls to "eradicate" various forensic disciplines as being insufficiently "scientific."

This presentation will impact the forensic science community by explaining the dangers to victims, defendants, and civil litigants if the move to "eradicate" various forensic disciplines succeeds.

When the 2009 National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward,* was published, how many of us realized that "the path forward" would involve a concerted effort to impose a wholesale ban on the use of well-established forensic disciplines? Calls by highly placed government officials to "eradicate" entire fields of evidence, along with well-funded attacks by defense groups, threaten to undermine the civil and criminal justice systems rather than to fix them. Jo Handlesman from the White House Science and Technology Office blasted forensic odontology and other disciplines, saying they were not based on science but relied on "gut reaction." She said, "These are the types of methods that must be eradicated from forensic science and replaced with those that come directly out of science."[1]

Any discussion must recognize that testimony by forensic dentists, although grounded in sciences like anatomy, histology, and dental medicine, is also based on the skill and experience of the forensic dentist, including his/her skill in pattern impression analysis. The same holds true for forensic pathology, forensic psychiatry, latent print analysis, and a host of other disciplines. None of these are bench sciences in which the same experiment always yields the same result. After all, we do not shoot volunteers at point-blank range to study gunshot wounds, or feed people increasing amounts of fentanyl to determine the lethal dose. Instead, we wait until they present at the emergency room or at the morgue and make observations that inform diagnoses and conclusions.

Any discussion must also accept that each bitemark is a unique event, as is every injury to a murder victim; every latent print is left under unique circumstances, as are footprints or tire tracks at a crime scene. Diagnoses of mental illness and its effect on criminal responsibility can be highly subjective and fiercely debated among experts. Ultimately, it all constitutes opinion, albeit expert opinion. How do we determine what comes directly from science, or what definition of science or evidence controls?

Defense counsel often seek to introduce the very kinds of evidence slated for extinction; this scorched earth approach affects everyone. Suspects often benefit from the threatened disciplines. Identification of one suspect exonerates another; forensic evidence provides proof of self-defense or consent. Post-conviction testing requests always seek proof that "some other dude did it." Careful thought must precede any move to eradicate forensic odontology. Many child abuse and fatality cases involve bitemark comparisons, where victims live with the perpetrator and DNA may be cleaned away or is simply not probative.

Although important lessons are learned from exonerations, decisions to eradicate 2016 forensics because of 30-year-old mistakes will have far-reaching negative effects. Newspapers only report plane crashes, not the overwhelming number of safe landings. With courts already equipped to handle opposing forensic theories through discovery, cross-examination, and experts for each side, it is far wiser to improve forensics rather than eradicate them.

Reference(s):

1. Handlesman J. International Symposium on Forensic Science Error Management – Detection, Measurement and Mitigation, Arlington, VA, July 20-24, 2015.

Forensic Odontology, Pattern Impression Analysis, Eradication

G23 Doyle — The Bitemark Case That Started It All

Roger D. Metcalf, JD, Tarrant County, Medical Examiner's District, 200 Feliks Gwozdz Place, Fort Worth, TX 76104; and Janice W. Klim-Lemann, DDS, 1802 Canyon Road, Redlands, CA 92373*

After attending this presentation, attendees will have a better appreciation of the history of the *Doyle* bitemark case.[1]

This presentation will impact the forensic science community by providing historical background to the area of bitemark analysis.

James A. "Jimmy" Doyle was arrested in west Texas, not far from Abilene, in December of 1952 for public intoxication. The night before Doyle's arrest, someone had broken into Oscar Peacock's grocery store, in the small town of Aspermont, and had stolen a number of items, including two bottles of whiskey and 13 silver dollars. In addition, a "large block of cheese" was found in the store, which "bore pronounced teeth marks." The cheese was sent for analysis by a novel-at-the-time procedure and was assessed by a Texas Department of Public Safety firearm and tool mark examiner, Mr. Taylor, as well as by Dr. Kemp, a dentist.

Mr. Doyle was charged with and convicted of burglary by a jury in Stonewall County. Mr. Doyle then applied for *certiorari* with Texas' highest criminal court, the Texas Court of Criminal Appeals, and the Court agreed to hear his case. Doyle claimed in error that the order from Sheriff Frazier for Doyle to bite into an example piece of cheese violated his right against self-incrimination because he had not received the mandatory warning required in Texas at the time (predating *Miranda* by more than a decade). The Court disagreed and the verdict was upheld. Doyle was Texas' first known bitemark case and the first known *reported* (in the legal sense) bitemark case in the United States.

This bitemark case is often cited by odontologists, but many may not know the interesting story "behind the scenes." The objective of this presentation is to provide some background history about the seminal bitemark case in United States forensic odontology and to discuss its subsequent ramifications. This presentation will provide greater understanding about the history of a very important case in the field of bitemark analysis and a greater appreciation of subsequent developments.

Reference(s):

1. *Doyle v. State*, 263 S.W.2d. 779, Tex.Crim.App. 1954.

Bitemark, Cheese, Odontology

G24 The Near-Tragic Results of a Misdiagnosed Bitemark by an Untrained Professional

Thomas V. Brady, DMD, 1823 Boston Post Road, PO Box 622, Westbrook, CT 06498*

After attending this presentation, attendees will learn to objectively evaluate a pattern injury based on the lesion(s) present, not on rash suppositions. Attendees will learn to diagnose the evidence independently and come to a reasonable opinion absent external pressure.

This presentation will impact the forensic science community by reviewing how the results of a rash diagnosis by a non-trained but important person may lead to a family tragedy and the possible permanent physical injury to a susceptible infant. Attendees have to maintain an independent, keen, trained eye on the evidence to avoid a miscarriage of justice.

A young mother became concerned over apparent lesion(s) on her 2-month old infant's leg. The mother took the infant to the child's pediatrician. The pediatrician could not diagnosis the lesions and sent the mother and child to Yale-New Haven Hospital for blood tests and further evaluation. Fearing the child had a genetic bruising disorder, the hospital admitted the infant. While being examined in the hospital, a medical department chief happened by, saw the lesions on the child, and immediately diagnosed the lesions as human bitemarks. To confirm his diagnosis, the department chief had a dental resident look at the child's leg injuries. The resident agreed with the senior physician.

This was all conducted with no swabbing for DNA, an eyeball view with no magnification, inadequate photos, no consultation with the family, and no opinion from a trained forensic odontologist, although several were available.

The hospital called the local police and the State of Connecticut Department of Children and Families, as mandated by law when abuse is suspected. The child was removed from the mother's custody pending further police and state investigation. A court date to determine whether to indict the mother for child abuse was set for 2 months and 11 days after the start of this ordeal.

Two days later, the mother was summoned to the hospital. Her infant was in severe distress. The mother was allowed to see her baby for two hours-a-day, was asked to breast feed the child, and to supply mother's milk for other times. Due to lack of hormonal stimulation and stress, the mother's milk production dried up after three weeks. The formula that the hospital provided caused severe constipation and dehydration. The child suffered severe bouts of stomach pain, had to have diuretics, and became malnourished.

During the course of their investigation, the local police consulted a forensic odontologist for his evaluation of the evidence. There were three possible human suspects and four small dogs in the house. Models of the humans were taken as well as pictures of the animal's dentition. The available pictures from the mother and the hospital were studied using the a image process software program, which was used to sharpen the images for further evaluation.

A thorough evaluation and a study of the totality of the scene, interviews with the suspects, and available evidence led to the determination that two of the dogs were the probable biters. The biting was not necessarily malicious on the dog's part. The child had a large attachment to her pacifier. As she suckled the pacifier, the attachment would move and the dogs would playfully nip at the attachments. It is also postulated that the smell of the child's apocrine glands, in the groin and leg area, attracted the dog's sense of smell with a familiar member of their family. The police determined that no crime had been committed, no charges were brought, and the court date was canceled. The dogs were removed from the house and 48 days after this ordeal started, the family was reunited.

After a legal case was brought against the hospital, a determination was made that the hospital would be liable for any developmental issues that may arise in the child. There is an eight-year period to evaluate the child's maturation before a final settlement is reached.

Bitemark, Untrained Diagnosis, Family Tragedy

G25 The Rise and Fall of Bitemark Matching and Bitemark Recognition: Blame It on DNA … or, What's Next?

Charles Michael Bowers, DDS, JD, Dental and Forensic Services, LLC, 2284 S Victoria Avenue, Ste 1G, Ventura, CA 93003*

After attending this presentation, attendees will better understand how meta-data review of legal case outcomes, multidisciplinary review, and media commentary, accompanied by objective empirical research and DNA methods present severe obstacles to forensic practitioners still relying on judicial stare decisis for their legitimacy.

This presentation will impact the forensic science community by allowing a more public look at these topics, which are omnipresent in social and print media.

Excessive expert disagreement in pattern analysis reflects poor reliability performance among bitemark analysis practitioners. Considerable odontological evidence was presented at the 2015 American Academy of Forensic Sciences (AAFS) Plenary Session that reinforced motivational bias as a contributing factor. Pre-trial and post-conviction DNA disagreement with prosecutorial bitemark opinions on the source, and the mere existence of skin patterns resulting from bitemarks, reflects poor validity in this AAFS/American Board of Forensic Odontology (ABFO) -accepted subset of forensic odontology.

Substantive research in the decades-long bitemark literature has failed to support human skin as an accurate pattern substrate. The National Academy of Sciences 2009 Report, Strengthening Forensic Science in the United States: A Path Forward, agrees with this fact, which conflicts with the few odontologists still practicing this field of forensic odontology. To date in the legal arena, the United States courts have been unable to keep up with the rise and fall of 40 years of stare decisis appellate holdings that accept bitemark matching and recognition. Some states' citations affirming this practice still use references that have become legally determined exonerations. This is despite the input of Frye, Daubert, Kumho, and judicial colleges on how to render opinions on what constitutes "science." This gap has very recently resulted in forensic reform advocates successfully advancing new legislation to educate and mandate courts to recognize and "weigh" (a notable non-science approach) empirical evidence-based "paradigm" shifts in forensic practice as being "new evidence." In a sense, politicians are creating their own piecemeal, state-by-state reform of forensics outside the hallowed halls of the practitioners and their committees.

Odontology Gap Analysis, Pattern Misidentification, Stare Decisis

G26 Bitemark Analysis and Comparison: Science, Observation, and Opinion

Thomas J. David, DDS, 1000 Johnson Ferry Road, Bldg H, Marietta, GA 30068; and Holland Maness, DMD*, 875 Union Avenue, Memphis, TN 38163*

After attending this presentation, attendees will learn the scientific rational behind bitemark analysis.

This presentation will impact the forensic science community by explaining the scientific literature that supports the underpinnings of bitemark analysis. The examiner may then observe and form an opinion regarding the patterned injury.

Many people believe that bitemark analysis is a single process; however, knowledgeable forensic odontologists understand that it is actually two processes, with the second predicated on the outcome of the first. The analysis is to determine whether the patterned injury in question is actually a bitemark. If so, then one proceeds to the second process — comparison of the bitemark to suspected biters. If the patterned injury is determined not to be a bitemark, or if there is insufficient evidence to make a determination, the process ends without a comparison. As a matter of fact, most patterned injuries that are analyzed never get to the comparison stage for the reasons outlined above.

Since the release of the National Academy of Sciences (NAS) Report in 2009, Strengthening Forensic Science in the United States: A Path Forward, there has been much criticism of the use of bitemark evidence. Some of these criticisms are valid and some are not. One of the most often heard critiques of bitemark evidence is that it is "not real science." In the sense that it is not measurable like DNA analysis and toxicology, there can be no doubt. Nevertheless, like many other forensic disciplines, it has scientific underpinnings along with observational opinions. Opinions given by medical examiners regarding the cause and manner of death would fall into the same category, because they are based on scientific principles that are combined with observations and experience to reach an opinion. In short, these opinions are not based on benchtop or laboratory science.

Bitemark analysis and comparison involves the use of scientific information, some of which is measureable. The initial stages of bitemark analysis usually include measurement of arch and tooth sizes. There is scientific data to support the use of human arch and tooth sizes in dental and orthodontic literature. In addition to measurement of arch and tooth sizes, histological examinations are sometimes used to determine whether an epidermal abnormality has subdermal hemorrhage and to determine relative aging of a patterned injury. All of these procedures are measureable scientific methods. The observational part of bitemark analysis and comparison is subjective and involves interpretation of arch and tooth shapes. Comparisons of suspected biters with the bitemark involve observation of consistencies and inconsistencies between the bitemark and the suspected biter. Inconsistencies are usually more significant than consistencies. An explainable inconsistency does not exclude a suspected biter; however, an unexplainable inconsistency is the basis for exclusion of a suspected biter.

The opinion part of bitemark analysis and comparison is a combination of measurement, observation, and experience. Experience does play a role in this part of this process, especially when deciding how much weight to give individual characteristics and distinct features of bitemarks. This part does not lend itself to scientific study because each injury is created in a different environment and cannot be directly compared to another case. The vast majority of opinions in bitemark analyses result in inclusion or exclusion of suspected biters. This may not have been the case during the time that most DNA exonerations occurred (prior to 1995), but forensic odontologists have recognized the limitations of their science. In addition, the American Board of Forensic Odontology (ABFO) has been proactive in correcting some of the problems with previous bitemark opinions. The ABFO has enacted or recommended the following changes within the past five years: (1) the blinding of bitemark evidence — the bitemark analyst should not collect the dental evidence from suspected biters; (2) the use of second opinions in bitemark comparisons; and, (3) the ABFO does not sanction the use of the strongest linkage opinion (the biter) in an open population.

In conclusion, bitemark evidence has significant value when used under the following circumstances: (1) the bitemark has substantial evidentiary value; (2) the population of suspected biters is relatively small; and, (3) the bite patterns of the suspected biters are distinctly different. The opinions that result from bitemark analysis and comparison can include suspected biters or exclude them. These opinions can provide valuable assistance in the determination of judicious outcomes for criminal suspects.

Bitemarks, Science, Opinion

G27 The Anatomy of an Aborted Retrial Involving Bitemark Evidence

Robert B.J. Dorion, DDS, Laboratoire S.J.M.L., Edifice Wilfrid-Derome, 1701 Parthenais, 12ieme, Montreal, PQ H2K 3S7, CANADA*

After attending this presentation, attendees will better understand the dated and improper use of photography, methods, materials, and techniques in an infanticide case.

This presentation will impact the forensic science community by demonstrating the effects of writing a proper, complete, detailed, expert witness report and by using approved guidelines as an essential component of judicial proceedings.[1,2]

In 2006, the body of a 3-year-old girl arrived unclothed at autopsy and no clothes accompanied the body. The child suffered a fractured skull, a healing fracture of the pelvis and wrist, liver laceration, broken ribs, spinal injuries, multiple bruises, abrasions, and lacerations, and more than 13 suspected human bitemarks, some of which were healing. The bitemarks were found on various parts of her anatomy, including the forearms, arms, finger, abdomen, mons pubis, buttocks, thighs, legs, and foot. The cause of death was established as multiple blunt force injuries with non-accidental trauma.

A pathologist and dentist were present at autopsy, and the prosecution hired two additional board-certified forensic dentists to review the case at a later stage. A male defendant was sent to trial in the case on charges of first-degree murder, aggravated sexual assault, and sexual interference principally based on the bitemark evidence. The case went to preliminary inquiry in 2010 and to trial in 2012. The initial board-certified forensic dentist testified for the prosecution at both events. The trial court judge acquitted the accused, and the Court of Appeal ordered a new trial on the basis of judicial error. There was no dental expert witness for the defense at the preliminary inquiry or at the trial. For both the preliminary inquiry and the trial, the case was considered a "closed population" event; however, for the retrial, further investigation discovered that more persons had been in contact with the child than had originally been thought, and thus it became an "open population" case. A forensic dental expert witness for the defense was appointed for the scheduled retrial in 2014.

The burden of proof in the jurisdiction in question belongs to the prosecution. This presentation will focus principally on the forensic elements and particularly the bitemark evidence leading up to this eight-year judicial marathon. The elements leading up to the retrial will be discussed. These elements include known and accepted facts that placed the accused in another place at the time of the child's death, pre-digital photographic conversion problems, the improper autopsy bitemark protocol, the improper use of dental materials and methods in evaluating the bitemarks, bitemark orientation issues, dental line-up issues, the improper use of dental casts for comparisons, the contradictory forensic bitemark prosecution expertise, and the DNA results.

In conclusion, the prosecution withdrew all charges against the accused the week before retrial. This case illustrates that a detailed defense expert witness report can at times halt judicial proceedings and prevent a potential wrongful conviction.

Reference(s):

1. Dorion R.B.J., ed., *Bitemark Evidence: A Color Atlas and Text*, 2nd edition. CRC Press, Boca Raton, Fl., 2011.
2. *American Board of Forensic Odontology Diplomates' Reference Manual*, January 2015 edition. American Board of Forensic Odontology,Inc.

Forensic Odontology, Evidence, Bitemark

G28 Bitemark Evidence — Part 2: Antemortem vs. Postmortem Bitemarks as Experimental Models

Robert B.J. Dorion, DDS, Laboratoire S.J.M.L., Edifice Wilfrid-Derome, 1701 Parthenais, 12ieme, Montreal, PQ H2K 3S7, CANADA*

After attending this presentation, attendees will better understand distortion of antemortem bitemarks vs. postmortem bitemarks. This analysis is crucial since distortion based on these two factors alone have not been compared.

This presentation will impact the forensic science community by informing attendees of the various potential problems involved in human bitemark interpretation, analysis, and comparison with suspect dentitions.

Porcine skin is considered as a representative model for the study of human bitemarks.[1-5] Error rates in human bitemark analyses have been calculated using the porcine skin model as a substitute for human skin.[6] A presentation at the 67th Annual Scientific Meeting of the American Academy of Forensic Sciences discussed the distortion potential of antemortem bitemarks.[7] This current study compares an additional 20 bitemarks inflicted by the same dentition (known biter) as antemortem bitemarks. Different parts of the porcine anatomy were bitten, including the neck, thorax, axilla, thigh, stomach, and back. The color photographs were lens and metrically corrected before comparison. Antemortem and postmortem bitemark photographs were taken at the time of bitemark infliction and on the third day postmortem. The latter scenario was chosen to mimic a potential real-life encounter of a Friday body recovery with a Monday autopsy. Attendees will have a rare opportunity to observe changes in the bitemark pattern from infliction to the third day of observation. The bitemarks were compared separately and serially. The same exercise was performed comparing the bitemarks with the dentition that created them.

Conclusions will be drawn from this exercise in order to minimize potential difficulties of interpretation, analysis, and comparison with a potential suspect dentition. The amount of distortion created in antemortem bitemarks will be compared to the amount of distortion created in postmortem bitemarks. This should clarify whether postmortem bites distort at the same rate as antemortem bites, and if postmortem bites should be used experimentally to mimic what would be expected from antemortem bites.

Reference(s):

1. Avon S.L., Wood R.E. Porcine skin as an *in vivo* model for aging of human bite marks. *J. Forensic Odontostomatology*. 2005;23:30–39.
2. Bush M.A., Miller R.G., Bush P.J., Dorion R.B.J. Biomechanical factors in human dermal bitemarks in a cadaver model. *J. Forens. Sciences* 2009;54(1):167–176.
3. Bush M.A., Miller R.G., Dorion R.B.J., Bush P.J. The Role of the Skin in Bite Marks, Part I: Biomechanical Factors and Distortion. *Proceedings of the American Academy of Forensic Sciences*, 60th Annual Scientific Meeting, Washington, DC. 2008.
4. Miller R.G., Bush P.J., Dorion R.B.J., Bush M.A.The Role of the Skin in Bite Marks, Part II: Macroscopic Analysis. *Proceedings of the American Academy of Forensic Sciences*, 60th Annual Scientific Meeting, Washington, DC. 2008.
5. Bush P.J., Miller R.G., Dorion R.B.J., Bush M.A. The Role of the Skin in Bite Marks, Part III: Microscopic Analysis. *Proceedings of the American Academy of Forensic Sciences*, 60th Annual Scientific Meeting, Washington, DC. 2008.
6. Avon S.L., Victor C., Mayhall J.T., Wood R.E. Error rates in bite mark analysis in an *in vivo* animal model. *J. Forensic Science International* 2010;201(3):45–55.
7. Dorion R.B.J. Bitemark Evidence. *Proceedings of the American Academy of Forensic Sciences*, 67th Annual Scientific Meeting, Orlando, FL. 2015.

Forensic Odontology, Evidence, Bitemark

G29 Unusual Bitemark Cases Demonstrate the Value of Bitemark Analysis

Richard H. Fixott, DDS, 3576 SW Valleyview, Redmond, OR 97756*

After attending this presentation, attendees will see the value of bitemark analysis in cases not linked to capital crimes. Attendees will be presented with casework with valid opinions based on the American Board of Forensic Odontology (ABFO) guidelines. Attendees will hopefully see the positive outcomes derived from rational analysis.

This presentation will impact the forensic science community by showing the value of bitemark analysis in atypical cases that were presented for analysis.

Several bitemark or alleged bitemark cases will be featured. The investigator is not always asked to compare an injury with a suspected dentition in order to include or exclude a suspect. Even limited information can assist law enforcement and attorneys with the case.

Case 1 — *He Said, She Said*: An alleged bite injury was under investigation. The odontologist testified for the prosecution in the first trial and for the defense in the second. The victim alleged the suspect bit her during an assault. The initial opinion was that the injury could not be absolutely ruled out as a bite. The opinion at the second trial was that the injury was such that meaningful comparison was not possible.

Case 2–*It's a Love Bite!*: An attorney asked if an injury could be differentiated as an amorous consensual bite or an assault as alleged by the victim.

Case 3–*Can you see any bites?*: Photos were submitted by the prosecutor to ascertain if any bites were evident. An 8-year-old child described a biting game with the mother's partner.

Case–*Oops!*: A possible domestic violence incident presents with an unusual bite injury. Police were called to a dispute. The husband stated that he often nibbled the wife's lip during kissing. As he kissed her, she pushed him away, causing an injury. The wife experienced copious bleeding, and the husband was arrested for assault.

Case 5–*Dog ear*: Who bit the ear — the owner or the best four-footed friend? An officer was called to an apartment residence by a person alleging that the neighbors were abusing their dog. The neighbors stated that the dog had gotten into the garbage and was upset about being placed in the shower. The dog had a cut on its ear and the investigating officer reported some blood in the shower. While discussing the ear injury, the owners mentioned how their grandfather used to bite his dog's ear to assert dominance. The case went to trial on third-degree animal abuse charges. The odontologist was asked for an opinion on whether the bite was human or animal.

Case 6–*How much evidence?*: Photos were reviewed to provide an opinion as to whether an injury was worthy of further study. On examination, it was difficult to determine maxillary from mandibular arches; however, some malalignment of teeth was evident, which may have been useful for including or excluding the suspect. The ABFO decision tree advised that no further workup was pursued, in spite of what the odontologist observed.

Discussion: These bitemark cases demonstrate the value and challenges of analyzing suspected bitemark evidence. A brief discussion will discuss "junk science" assertions against forensic odontology and the call for the "eradication" of bitemark testimony.

Bitemarks, Animal Abuse, Domestic Violence

G30 Assessing the Reliability of Measurements of Human Dental Casts Using an Intraoral 3D Scanner

Mithun Rajshekar, MFSc, 2/67 Olinda Grove, Mt Nelson, Hobart, Tasmania 7007, AUSTRALIA*

After attending this presentation, attendees will better understand using technological advances such as an intraoral 3D scanner in forensic investigations of bitemarks.

This presentation will impact the forensic science community by providing analytic results of a study in which measurements of dental landmark features that were made using hand-held intraoral 3D scanners are validated for their reliability and repeatability against measurements that were made using a conventional hand-held digital caliper. This study establishes the hand-held intraoral 3D scanner as a reliable tool that can be used to measure landmark dental measurements to be applied in the forensic analysis of dental evidence.

Background: Dental casts have been used to study dental anatomy and surrounding structures for research and therapeutic purposes, but their preparation is time-consuming and the procedure causes discomfort to subjects. In the forensic investigation of confirmed bitemarks, dental casts are compared to bitemarks found on victims. The materials currently used to make dental casts undergo structural and chemical changes while setting; consequently, the casts may not be completely accurate. Intraoral 3D scanning of dentition has the potential to provide a fast, accurate, and non-invasive method of recording dental information.

Goal: The goal of this study was to assess the reliability of measurements of human dental casts obtained with a portable intraoral 3D scanner that was appropriate for field use in forensic investigations.

Methods: Two raters each measured 84 tooth and 28 arch features of 50 sets of upper and lower dental casts. The first measurements used digital hand-held calipers, whereas the second measurements used the 3D measuring software provided with the Zfx IntraScan Intraoral 3D scanner to obtain 3D images of the digital dental casts. The measurements were repeated at least one week later. Reliability was quantified by comparison of means for test-retest, rater-rater, and method-method differences, regression of differences on covariates for factors influencing measurement error, and calculation of Intra-Class Correlation Coefficients (ICC) and Standard Errors of Measurement (SEM).

Results: The differences in the two measurements were small and did not vary with characteristics of the casts. Intra-rater ICC (3, 1) and SEM were 0.99 (95% CI, 0.98-0.99) and 0.0612, respectively, for the intra-oral 3D scanner, and 0.99 (95% CI, 0.99-1.00) and 0.0127 for the digital hand-held caliper, respectively.

Conclusion: The intra-oral 3D scanner provides measurements of features of human dental casts that are reliable and have comparable reliability to those made by conventional digital hand-held calipers. A future direction of this research is to confirm the reliability of measurements of non-human dentition.

Bitemark Analysis, Dental Evidence, Forensic Odontology

G31 Morphoanalysis of Bitemarks

Charles E. Georget, PhD, 5 Rue Voltaire, Amboise 37400, FRANCE; and Aime Conigliaro, MA, Fort de Rosny, 1 boulevard Théophile Sueur, Rosny sous-bois 93110, FRANCE*

After attending this presentation, attendees will better understand the interest in using specific softwares to create overlays and to superimpose 3D images of bitemarks with those of the dental arches of the alleged biter.

This presentation will impact the forensic science community by demonstrating that bitemark analysis must be reliable at all levels. This study is a continuation of research that began several years ago regarding bitemark expertise in the forensic odontology department of the Forensic Science Institute of the French police force.

The identification of bitemarks must enhance the scientific aspect of expertise. There is no longer any reason to compare simple overlays created from photographs using software, which implies the operator's subjectivity. 3D digital imaging enables superimposition of overlays and virtual penetration into soft tissues, which leads to more accurate expertise. Accurate overlays can be generated by software that executes close cuts. This automated clipping enables a more reliable illustration of the contours of the teeth. Different experts were instructed to make identical overlays from the same dental arch. This technique eliminates the claims of lawyers and magistrates regarding the accuracy of drawings submitted for examination. The examination of virtual penetration of teeth into the tissues is performed using the same software that brings the analyzed 3D elements closer. In the long run, this examination should enable comparison of the bitemark morphology of the victim with the morphology of a virtual bitemark experimentally realized.

Materials and Methods: The use of a laptop is necessary and sufficient to perform real-time 3D image analysis and reconstruction and to store data and software programs. An optical camera is linked to this laptop by a USB port. The software is used to visualize the dental arches of a suspect and the bitemarks on the victim. The camera is used to scan the bites on the body of the victim and the teeth or dental casts of the suspect. Then, virtual cuts are made, cropped, and a superimposition is performed by placing overlays on each workable mark on the skin of the victim. These files can be sent/shared in Stereolithography (STL) or Polygon (PLY) files for further analysis.

Conclusion: The analysis of human bitemarks is often controversial in both national and international courts. Only an objective analysis using new digital technologies and secure protocols can make this type of expertise credible. Morphoanalysis of bitemarks using the materials and method described in this study represents real progress in the field of forensic odontology.

Bitemarks, 3D Digital Camera, Overlay

G32 Dental Identification Challenges Using World War II Military Dental Records: Tarawa, 2015

Corinne D'Anjou, DMD, 222 rue l'Espérance, Saint-Lambert, PQ J4P1Y2, CANADA; David R. Senn, DDS*, University of Texas HSC San Antonio, 7703 Floyd Curl Drive, Mail Code 7919, San Antonio, TX 78229-3900; and James F. Goodrich, BDS*, 390 French Pass Road, RD 4, Cambridge, Waikato 3496, NEW ZEALAND*

After attending this presentation, attendees will be better informed regarding the identification of recently recovered United States Marine Corps and Navy personnel who died during the World War II Battle of Tarawa.

This presentation will impact the forensic science community by illustrating that forensic odontologists can assist in the identification of skeletal remains from a different time period, when the only available antemortem dental information consists of dental records and charts without dental radiographs.

The Battle of Tarawa occurred in the Pacific Theater of World War II from November 20 to November 23, 1943. It took place at the Tarawa Atoll in the Gilbert Islands. Nearly 6,400 Japanese, Koreans, and Americans died in the fighting, the majority on the island of Betio. The remains of 35 individuals were recovered from a previously undiscovered post-battle cemetery on Betio Island, Tarawa Atoll, in June 2015. Anthropological examination results indicated that they were males of European or mixed-European ancestry, and the remains were associated with many uniform and equipment items consistent with United States military personnel.

Antemortem military dental records were examined for 315 unrecovered service personnel presumed to have died during the battle. The dental remains were examined, photographed, and radiographed. Dental coding computer software was employed to aid in postmortem and antemortem data comparison. Dental age estimation techniques were applied to the remains. The dental remains were compared to OdontoSearch databases to establish population incidence of dental patterns for the deceased. At time of writing, 17 positive identifications have been made from odontological comparisons, 6 comparisons are classified as "probable," 6 are classified as "not-excluded," and 1 individual had no associated oral, facial, or dental remains. Additional antemortem dental records are reportedly available and forthcoming. Comparisons of those new records with current databases may yield additional results.

The comparison of postmortem evidence with only antemortem records is not a common practice in forensic odontology; however, in this case, several factors combine to allow a high degree of confidence that dental identification can be achieved within a limited population. The four factors include: (1) comprehensive and detailed written and graphical antemortem military dental records; (2) the quality of the excavation, recovery, and processing of the remains; (3) good preservation of the skeletal and dental remains; (3) the relative distinctiveness of certain individual dental patterns; and, (4) the application of multiple supporting techniques.

Dental Identification, World War II, Forensic Odontology

G33 The Trabecular Bone in Identification — Algorithms and Fractal Analysis

Sylvain Desranleau, DMD, Ordre des dentistes du Québec, 800 Boul René-Lévesque Ouest, Montreal, PQ H3B 1X9, CANADA; and Robert B.J. Dorion, DDS, Laboratoire S.J.M.L., Edifice Wilfrid-Derome, 1701 Parthenais, 12e étage, Montreal, PQ H2K 3S7, CANADA*

After attending this presentation, attendees will be aware of new information regarding the use of mandibular trabecular bone patterns to establish positive identification.

This presentation will impact the forensic science community by establishing a method of calculating the significance of mandibular trabecular bone patterns to arrive at a positive identification.

According to the University of California, Berkeley's orthopedic biomechanics research, the trabecular bone can be classified as a porous cellular solid, consisting of an irregular 3D array of bony rods and plates called trabeculae, which are composed of a calcified matrix. Bone marrow fills the pore spaces. Because all free bone surfaces are covered with bone cells, bone is a living tissue that is self-healing and has the ability to adjust its morphology in response to changes in its mechanical environment, which is the so-called but poorly understood phenomenon of bone remodeling. As such, the mechanical complexity of this two-phase biological tissue surpasses any engineering material, making it a fascinating subject of study, regardless of clinical applications.

The process of dental identification compares postmortem to antemortem data. It involves the analysis of the following factors: the presence and absence of teeth; crown and root morphology and their interrelationships; evaluation of the periodontal status; the type and extent of restorative, endodontic, fixed, removable, and implanted materials; tori and sinus configuration; anomalies and pathologies of teeth and bone; and, trabecular pattern morphology.

Few studies have been conducted on the statistical reliability of trabecular bone patterns for identification purposes. Some of these studies utilize algorithms, a mathematical expression that produces the answer to a question or the solution to a problem in a finite number of steps. Other studies perform fractal analysis, which involves assigning a fractal dimension or other fractal characteristic to a dataset. The theoretical dataset, pattern, or signal can include natural geometric objects, sound, market fluctuations, heart rates, digital images, molecular motion, and networks. There are several approaches for fractal analysis of odontological data. Fractal geometric techniques and methodological principles can be applied to study the trabecular bone. Radiographs can be analyzed to assess trabecular bone structure and predict elastic modulus and strength. Digital image analysis can be applied to study cadaver mandibular trabecular bone patterns. Fractal dimension and lacunarity analysis can be applied to dental radiographs and periapical radiographs, and morphodigital techniques can be applied to the mandibular trabecular bone in panoramic radiographs. The tile-counting method can be used for fractal analysis of mandibular trabecular bone. Particle counting methods can be applied to determine the anatomical variations of trabecular bone structure in intraoral radiographs. The trabecular bone can be analyzed using site-specific fractal values calculated from cone beam Computed Tomography (CT) images. The fractal dimension can be determined for the mandibular trabecular bone measured on digital and digitized images.[1-11]

As a continuation of the research project The Trabecular Bone in Identification, this current research focuses on algorithms and fractals as an aid and tool for trabecular bone pattern morphometric analysis and comparison.[12,13] This could lead to a revolutionary approach in handling human trabecular jaw bone patterns for identification and could be particularly practical in mass disaster situations involving large numbers of edentulous victims and/or fragmented remains.

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Trabecular Bone, Algorithms, Fractal Analysis

G34 Paradise Ablaze 6-2-6: Dental Identification of Charred Human Remains

Judy Y. Marshall, DMD, Marshall Family Dentistry, 3443 Tamiami, Ste F, Port Charlotte, FL 33952*

After attending this presentation, attendees will better understand the utility of dental identification of six charred human remains recovered from two house fires, which occurred at a six-year time interval.

This presentation will impact the forensic science community by demonstrating the durability of teeth to withstand the temperatures of house fires and by manifesting the value of dental identification of charred human remains recovered from two house fires which occurred on a barrier island in southwest Florida.

On Saturday, April 25, 2009, at approximately 2:30 a.m., a house fire erupted on Privateer Road on Palm Island, FL. An island volunteer fire truck and county fire boats responded, but the home was destroyed. Four charred bodies were recovered from the scene, and each body maintained dental evidence that survived the temperatures of the fire. The medical examiner requested that the forensic odontologist conduct dental examinations for the purpose of identification. Positive identification of three of the four bodies was accomplished using dental records.

Six years later, on May 24, 2015, at approximately 2:30 a.m., a house fire erupted on Gulf Shore Boulevard on Palm Island, FL. One island fire truck responded and additional emergency equipment and responders were ferried to the island. By the time fire trucks arrived, the home was engulfed in flames and was completely destroyed. Two charred bodies recovered from the scene were transported to the office of the medical examiner. The forensic odontologist performed dental examinations for the purpose of identification, and both bodies were positively identified using dental records.

The barrier islands of southwest Florida support secluded second homes and have beautiful beaches, spectacular sea shells, and sensational sunsets; however, this pristine paradise is not immune to disaster when house fires erupt and paradise is set ablaze.

This presentation will demonstrate the survivability of dental evidence in severely burned human remains recovered from barrier island house fires. Antemortem and postmortem radiographs of the decedents will be presented. The value of dental identification in charred human remains when antemortem dental records are available for comparison will be apparent in these case presentations.

Dental Identification, Charred Human Remains, Dental Evidence

G35 You Mean You Made an Identification Using What?

Mary Shields, DMD, 904 Lily Creek Road, Ste 101, Louisville, KY 40243; and Mark L. Bernstein, DDS, University of Louisville School of Dentistry, Dept of Diagnostic Sciences, Health Sciences Center, Louisville, KY 40292*

After attending this presentation, attendees will have a better understanding of typical and atypical presentations of the styloid process and how this could help with a positive identification of human remains.

This presentation will impact the forensic science community by providing an understanding of how anatomical variance of the styloid process can lead to a positive identification.

Introduction: Non-dental anatomical findings occasionally assist in dental identification. Examples include the use of frontal sinuses, palatal rugae, tori, and other bony pathosis. A case is presented in which a distinctive styloid process served to upgrade the confidence in a dental identification. After attending this presentation, attendees will be able to appreciate the value of finding an uncommon anatomical variance and its use in reaching a positive identification. Attendees will better understand atypical presentations and the Langlais Type Classification system used to categorize the degree of elongation and configuration of the styloid processes, which may be classified as Eagle Syndrome in a symptomatic patient.

Case Report: The nearly skeletal remains of a suicidal hanging victim found in a remote area in Carroll County, KY, in March 2015 were suspected to be that of a man reported missing the previous September. A dentist was located who treated the putative victim on two visits, one during which radiographs were made and another that recorded the extraction of tooth #31. The radiographs confirmed the presence of three composite restorations and several missing teeth, allowing a directed identification. The finding of an intact styloid process measuring 5.1cm proved to be the most distinctive characteristic, allowing for a more confident identification.

Discussion: The styloid process is rarely mentioned as a determinant of identity in forensic dental examinations because it is liable to be seen only in skeletal cases and is vulnerable to postmortem fracture and loss. Also, it must be compared to an antemortem radiograph that features this structure, typically a panoramic or cephalometric image. A normal styloid process measures <2.5cm.[1] Styloid processes (including those elongated due to calcification of the stylohyoid ligament) larger than 3cm are present in 4%-10% of the population; however, fewer than 5% of those cases are noted *in vivo* due to asymptomatic presentations. This low incidence constitutes a distinctive feature. Additionally, tortuous shapes, radiolucencies, and articulation variances add to the individuality of the finding. Elongated styloid processes with or without ligament calcification have clinical relevance and dentists should be aware of this. Pain when swallowing or turning the head due to impingement of the pharynx from an elongated styloid is called Eagle syndrome.

Significance: An elongated or distorted styloid process observed in skeletal remains can aid the forensic dentist in identification. Likewise, this finding in an antemortem radiograph can prompt the acquisition of comparative postmortem images in non-skeletal remains.

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Identification, Styloid Process, Eagle Syndrome

G36 Accuracy of the Cameriere's Method on Age Estimation on the Libyan Population

Ashref A.K. Dardouri, MSc, University of Huddersfield, Queensgate, Huddersfield HD1 3DH, UNITED KINGDOM; Roberto Cameriere, Via Don Minzoni 9, Macerata, ITALY; and Stefano Vanin, PhD, Queensgate, Huddersfield HD1 3DH, UNITED KINGDOM*

After attending this presentation, attendees will have novel information about age estimation using the Cameriere's method in the Libyan population with specific attention to the possibility of distinguishing people older or younger than 18 years of age, which is considered the legal age to be considered an adult in several countries.

This presentation will impact the forensic science community by demonstrating the accuracy of the Cameriere's method for age estimation in an area of the world where insufficient information on this topic is available. The Northern coasts of Africa play an important role in emigration from Africa to Europe. The availability of an accurate method to distinguish children from adults is fundamental from a legal and forensic point of view.

Since 2000, age estimation of living adults has become increasingly common in civil and criminal cases to address problems concerning the age of children in cases of adoption, criminals who refuse to provide their age, issues related to immigration and asylum requests to foreign countries, assessment of the capability of being imputable, prosecuting pedophilia, child pornography, and pensionable age for adults.

Estimating the age of a living person often requires an integrative approach that involves anthropology, forensic dentistry, and radiology. Human identification and aging using dentition have been well established in the forensic field, and several methods based on changes that occur in teeth during aging have been developed. The goal of this presentation is to validate, for the Libyan population, Cameriere's method based on 3rd molar analysis (I_{3M}). For this purpose, a sample of people from Tripoli, the capital city where different ethnic groups are represented in the population, was analyzed.

Panoramic radiographs (Orthopantomograms (OPTs)) of 420 Libyan people from different ethnic backgrounds, aged 14 years to 22 years, were analyzed. The samples were obtained directly by digital radiological technology and collected during January, February, and March of 2015. OPTs had been performed for clinical reasons, and consent to use them for research purposes was obtained from the patients or relatives for under-age children. Panoramic X-ray images with lost or extracted single-rooted teeth, fillings, crown restorations, severe caries, or other abnormal dental anatomy, which may have caused difficulty with the measurements, were excluded from this analysis. A total of 307 OPTs (163 females and 144 males) were finally examined. The results indicate that male and female I_{3M} values <0.08 are exclusively associated with individuals older than 18 years of age, whereas I_{3M} values >0.08 have a misidentification rate of 9%-11% (individuals older than 18 years of age can be classified in the younger group). An increase of the cut-off value to 0.09 does not affect the possibility of identifying individuals older than 18 years of age, and reduces misidentification to 2%-3%.

In conclusion, Cameriere's method correctly distinguished individuals under the age of 18 years when applied to the Libyan population. Only a small number of older individuals are incorrectly included into the younger group (<18 years), whereas none of the younger individuals is incorrectly included in the older category. This conclusion has important applications in the Libyan forensic context, in which the necessity for reliable and accurate age estimation techniques has never been greater than in the last five years, primarily due to armed conflicts within the country. The lack of a validated method for age estimation in the Libyan population is fundamental, both in world crime investigation and in emigration/immigration control at the national and international level.

Age Estimation, Teeth, Cameriere Method

G37 Restorative-Era Identification of a Severed, Embalmed Head

Raymond G. Miller, DDS, 122 Covington Road, Buffalo, NY 14216; Mary A. Bush, DDS, SUNY at Buffalo, B1 Squire Hall, 3435 Main Street, Buffalo, NY 14214; and Peter J. Bush, BS, SUNY at Buffalo, South Campus Instrument Center, B1 Squire Hall, S Campus, Buffalo, NY 14214*

After attending this presentation, attendees will better understand the use of dating restorative materials in postmortem profiling.

This presentation will impact the forensic science community by increasing awareness of variations in restorative materials and techniques in different eras and applying this information in establishing a postmortem profile.

The practice of dentistry has undergone dramatic changes in the past 60 years with the introduction of adhesive restorative materials. These products have improved the esthetics, strength, durability, and predictability of restorative dentistry. This is especially true of materials used to restore anterior teeth. Manufacturers have introduced products with varying properties and chemical makeup, and these products have evolved over time. These properties can be used by the forensic odontologist as a marker of different eras. This information is useful when establishing a postmortem profile of an individual. Thus, an approximate time frame can be established by analyzing and identifying the materials and probable techniques applied in a victim's restorative profile. Essentially, in order to have received a specific type of treatment with specific materials, a victim would have needed to undergo the restorative procedures during a certain period of time when these techniques and materials were commonly available.

In December 2014, an embalmed head was discovered roadside in a municipality in Pennsylvania. A forensic dental exam was performed on the recovered specimen by the local forensic dental consultant and reported to the coroner. In another municipality approximately 90 miles away, a mausoleum was found disturbed with the door ajar. Further investigation discovered a headless body. Questions were raised regarding if the embalmed head belonged to the headless body. The date of interment in the mausoleum was known. There was little information available on the embalmed head. The postmortem dental profile of the head revealed dental treatment that was not consistent with the level of treatment available at the time the body was interred. The dental treatment on the severed head appeared to postdate the type of care available at the time the headless body had died because the treatment used advanced materials and techniques. The consulting forensic odontologist in Pennsylvania requested assistance from the Laboratory for Forensic Odontological Research at the State University of New York at the Buffalo School of Dental Medicine to evaluate the restorative materials in the severed, embalmed head. The Pennsylvania State Police delivered the head to the University in January 2015 for analysis. The goal was to analyze the restorations, determine elemental composition and other properties, and possibly estimate the era or period the restorative materials were available and in use. Cone beam and conventional radiography and Scanning Electron Microscopy with Energy Dispersive Spectroscopy (SEM/EDS) were utilized for the analysis. Radiopacity and elemental composition were also evaluated.

The information obtained through the laboratory analysis, in addition to the postmortem dental profile of the embalmed head, determined that there was not a relationship or link between the severed head and the headless body. The dentistry of the embalmed head postdates the death of the interred body. Further investigation is required to locate the missing head and body.

Dental Profile, Restorations, Analysis

G38 Annapolis Mansion Fire — January 2015: Utilizing Dental Identifications

Patrick A. Murray, DDS, 15200 Shady Grove Road, Ste 340, Rockville, MD 20850; Ali Behnia, DMD, 9707 Key West Avenue, Ste 130, Rockville, MD 20850; Pete N. Nickolas, DDS, 114 E Main Street, Westminster, MD 21157; and Warren D. Tewes, DDS, Office of the Chief Medical Examiner, State of MD, 108 Bakers Lane, Queenstown, MD 21658-1101*

After attending this presentation, attendees will recognize the benefits of dental identifications in horrific fire disasters. Attendees will also appreciate the steps utilized in making the dental identifications.

This presentation will impact the forensic science community by sharing the details of the horrific fire in Annapolis, MD, and the identification of the six victims of that fire.

On January 19, 2015, a mansion in Annapolis, MD, was completely destroyed by fire and killed six family members. The Medical Examiners' Dental Identification Team (MEDIT), a component of the Maryland Responds Medical Reserve Corps to the Office of the Chief Medical Examiner (OCME) in Baltimore, provided subject matter expert assistance. The Maryland Responds forensic odontologists liaised with the OCME Chief Forensic Odontologist.

Antemortem family dental radiographs and written records of the victims were collected from treating dentists by law enforcement investigators and delivered to the OCME. The MEDIT members examined and collated the dental records into six single documents that illustrated the best dental representation for each victim prior to the tragedy. Over the next six days, victim remains were methodically recovered from the extinguished inferno to preserve evidence. The remains were delivered to the OCME per protocol. Postmortem dental examination and radiographic images produced six postmortem records. These new records were compared to the previously prepared antemortem records.

There are three legal methods of human identification, including antemortem/postmortem fingerprints, DNA, and dental comparisons. The goal of each method is to categorize and exclude all non-match victims, yielding a single remaining victim that matches an antemortem record. Fingerprints can be quickly degraded, and DNA often requires extensive analysis and prohibitive costs. The rapid acquisition of antemortem dental records enables a single positive identification to be rendered in a timely fashion for the grieving family.

"Closure" is a term frequently used by the public and media in these circumstances, but it is rarely heard within the forensic community and by families. The forensic community is more focused on achieving a 100% correct result and completing their work in a timely fashion. Families are wounded forever by these tragedies, but they can rest assured that the human remains released by the OCME are in fact their loved ones ready for burial.

Consequently, members of the Maryland MEDIT, like other Medical Reserve Corps specialty volunteers, experienced a sense of privilege in their swift contribution in completing the dental identifications in order to resolve this case. The families are now able to move forward with funerals and burials. Hopefully, sometime in the future, the families will experience healing and resolution with the gift of time.

Annapolis, Dental IDs, Fire

G39 Identification of Decomposed Human Remains Found in a Septic Tank

Donna J. Hellwinkel, DDS, 4555 Saddlehorn Drive, Reno, NV 89511*

After attending this presentation, attendees will be better informed regarding how a medical examiner's office and a law enforcement agency used scientific methods and old-fashioned detective work to identify decomposed human remains found in a septic tank.

This presentation will impact the forensic science community by illustrating the five forensic methods (visual, fingerprints, anthropology, DNA, and dental) commonly used in human identification.

On August 27, 2013, partially skeletonized human remains were found when a septic tank service company routinely drained a residential septic tank in Storey County, NV. These remains were brought to the Washoe County Medical Examiner's Office (WCMEO) in Reno, NV, where an autopsy and postmortem data collection were performed. Fingerprints were taken and analyzed. Forensic anthropology and forensic odontology examinations were performed.

Storey County law enforcement checked missing person rosters and submitted a National Crime Information Center (NCIC) search. No information regarding the identity of this unidentified person was obtained. It was not until a WCMEO death investigator performed a Google® search on the design and logo of a medallion recovered with the body that a lead on this person's identity was generated. Law enforcement expanded on this lead. More information was gathered, including United States Navy dental records. When these records were compared to the results of the forensic dental examination, a possible dental identification was obtained. Law enforcement located a biological relative of this individual and DNA was obtained from a section of the unidentified person's femur and compared to that of the potential biological relative. This culminated in positive identification of a man who had been reported missing in 1980.

A septic tank system is a privately owned, on-site sewage treatment plant. Septic tanks are usually located in rural areas lacking connection to a municipal sewer system. The naturally occurring populations of bacteria in the tank break down organic solids and dissolved chemicals in waste water. Corrosive chemicals like sulfuric acid, hydrogen peroxide, and formaldehyde are added when drain pipes become clogged. Sludge accumulation in the tank is reduced by having the tank pumped every three to five years. The effects of such an environment on a human body for more than 20 years will be discussed.

This unique identification case illustrates the importance of combining detective work and forensic science when endeavoring to identify severely decomposed human remains.

Forensic Science, Forensic Odontology, Dental Identification

G40 Dental Morphoanalysis and Identification of Monozygotic Twins

Aime Conigliaro, MA, Fort de Rosny, 1 boulevard Théophile Sueur, Rosny sous-bois 93110, FRANCE; and Charles E. Georget, PhD, 5 Rue Voltaire, Amboise 37400, FRANCE*

After attending this presentation, attendees will better understand that the characteristics of monozygotic twins' teeth can provide useful information for the identification of skeletons or burned or decomposed victims.

This presentation will impact the forensic science community by demonstrating why no usable dental clue should be overlooked even though the genetic results provide more reliable results.

Dental morphoanalysis studies the shape of the teeth and particularly the shape and position of grooves and cusps located on the occlusal surfaces. The scope of morphoanalysis is limited to specific cases. This presentation describes a case involving comparative study of the teeth of monozygotic twins.

Materials and Methods: The study of dental characteristics of heterozygous and monozygotic twins is based on the examination of plaster casts made from dental prints. These models are observed with the naked eye, then with a magnifying glass. Finally, they are photographed to record and analyze the data. The teeth are examined in two phases and at two levels: (1) study of the general morphology takes into account the shape of the dental arches and the position and number of teeth on the arch and determines the type of occlusion; and, (2) morphodental analysis is performed with a magnifying glass and photographic enlargements. Data are obtained from the two studies, one that is descriptive and one that involves dimensional measurements.

Results: Although heterozygous twins do not display comparable usable elements, monozygotic twins do. The observations allowed for the identification of some key points. In monozygotic twins, the descriptive study of occlusal surfaces provides considerable usable information, even if some treatment or certain pathologies disrupt the analysis. For each tooth, several characteristics are studied for comparison. For molars and premolars, the following characteristics are observed: (1) cusps whose arrangements vary depending on the treated tooth; (2) axial position of the edge of the cusp compared to the transverse edge; (3) major and minor grooves; (4) the form of primary and secondary dimples; and, (5) tubers and all existing defects.

For canines and incisors, the shape of the palatal surfaces, canine tips, and incisive edges are essential and discriminating characteristics. Dimensional measurements involve the study and measurement of all teeth with dividers. These measurements include the mesiodistal diameter, buccolingual diameter, and height of the collar at the free edge of each tooth.

Conclusion: In the absence of antemortem records of deceased and unidentified monozygotic twins, the comparative study of dental casts can discriminate the twins among a given population. This study provides detailed information on each individual twin once isolated from the rest of the population.

Twins, Identification, Morphoanalysis

G41 Photographically and Radiographically Observed Dental Evidences Validated for Human Identification Purposes

Nikolaos Angelakopoulos, KU LEUVEN, Ijzerenmolenstraat 24/214, Heverlee 3001, BELGIUM; Guy Willems, PhD, Katholieke Universiteit Leuven, School of Dentistry, Kapucijnenvoer 7, Leuven B-3000, BELGIUM; Ademir Franco, MSc, Katholieke Universiteit Leuven, Kapucijnenvoer 7, block a, Leuven, BELGIUM; Steffen Fieuws, PhD, Kapucijnenvoer 7, Leuven B3000, BELGIUM; and Patrick W. Thevissen, PhD, KU Leuven, Dendermondsesteenweg 483, Sint-Amandsberg, Oost Vlaanderen B-9040, BELGIUM*

After attending this presentation, attendees will better understand which oral evidence(s) registered in photographic and radiographic dental records provide the highest performance to identify a subject for forensic odontological identification purposes.

This presentation will impact the forensic science community by illustrating how specific tooth- and dentition-related oral identifiers based on photographically and radiologically observed dental features can establish forensic odontological identifications.

The use of preventive dentistry is a rising trend worldwide because patients are more aware of oral health. Consequently, a reduced number of dental identifiers is expected in the near future due to treatment interventions.[1,2] From a forensic view, the reduced number of dental restorations means that human dental identifications will be necessarily founded on the oral and dentomaxillofacial morphological, positional, and pathological identifiers. The present study had two goals: (1) to register specific tooth- and dentition-related oral identifying variables on intraoral photographs, panoramic radiographs, and cephalometric radiographs; and, (2) to detect the oral identifying variable that was most useful for identifying the correct individual for each considered registration technique.

Retrospectively, a reference set of 1,727 unique subjects was collected for which a standardized set of five intraoral photographs, a panoramic radiograph, and a cephalometric radiograph were registered at the same time. Specific tooth- and dentition-related oral identifiers were collected and scored from the sets of photographs, panoramic radiographs, and cephalometric radiographs. Two observers each scored 895 and 832 subjects, respectively. For 308 subjects of each group, scores also were established by the other observer. These sets of 308 subjects were referred to as the inter-observer sets. For each of the scored identifiers, the distance was quantified between the scores from the inter-observer set and each of the scores in the reference set. The number of subjects in the reference set with a distance at least as small as the correct subject was referred to as the potential set and was expressed as a percentage of the reference set. The mean potential set was reported for each of the registration techniques separately.

For the photographic information, the panoramic information, and the cephalometric information, the number of molars (34.6%), the number of missing teeth (42.0%), and the number of displaced teeth (59.9%) were the most useful variables in identifying the correct subject, respectively. The shape of the central incisors was the best tooth-related identifying variable for the photographic and panoramic registrations (58.8% and 75.8%, respectively). By contrast, no best tooth-related identifying variable could be detected for the cephalometric registration. For each of the three types of registration techniques, the most identifying variables differed but were consistently dentition-related.

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Dental Identifiers, Oral Photography, Dental Radiology

G42 Teeth and Fire: Forensic Analysis of Teeth and Dental Material Exposed to Fire

Joe Adserias, DDS, PhD, C/ Balmes 62, Barcelona, SPAIN; Sara C. Zapico, PhD, Smithsonian Institution, Dept of Anthropology, NMNH, MRC 112, 10th & Constitution Avenue, NW, Washington, DC 20560; Luis L. Cabo, MS, Mercyhurst University, Dept of Applied Forensic Sciences, 501 E 38th Street, Erie, PA 16546; Steven A. Symes, PhD, Mercyhurst University, 501 E 38th, Erie, PA 16546; Douglas H. Ubelaker, PhD, Smithsonian Institution, Dept of Anthropology, NMNH, MRC 112, Washington, DC 20560; and Dennis C. Dirkmaat, PhD, Mercyhurst University, Dept of Applied Forensic Sciences, 501 E 38th Street, Erie, PA 16546*

After attending this presentation, attendees will better understand the macroscopic changes undergone by burned teeth as well as some of the techniques that can be applied in their analysis.

This presentation will impact the forensic science community by illustrating the usefulness of integrating the observations obtained from the analysis of burned dental remains from different contexts, such as forensic cases, commercial cremations, and experimental studies.

Burned skeletal remains are associated with different scenarios of forensic interest, including traffic accidents, explosions, domestic fires, plane crashes, or natural disasters. Fire also is used to destroy forensic evidence in criminal cases, and commercial cremations can raise forensic questions related to identification or commingling issues. Different techniques are useful in the analysis of burned remains, including macroscopic, X-ray, and molecular DNA analyses. Macroscopic analysis focuses on parameters such as color changes, fire-related fracturing, detection of dental treatments and prostheses, and anatomical traits useful for identification. Radiographic analysis detects antemortem dental treatments, fire-related changes in the physical properties of dental materials, and non-visible dental fractures. The integration of these analyses can help to detect and select suitable or more promising samples for DNA analysis.

First, the results of 40 commercial cremations from the Memora Funeral Home (Salt, Girona, Spain), which were examined and documented using standardized protocols before and after cremation, are presented. The retort conditions (temperature and time of exposure) were essentially the same for all individuals included in the study. Commercial cremation is one of the more destructive treatments of human remains. This process involves burning the body until all organic materials are destroyed by heat, followed by pulverization of the burnt remains before returning the ashes to the family. In spite of the destruction of some dental structures during the burning process, this presentation illustrates how analysis of cremains before pulverization can still provide relevant information for comparison with dental records, including dental prostheses and typical heat-induced dental alterations.

Second, the results of a laboratory study directed at evaluating the macroscopic changes induced on teeth by heat exposure under controlled time and temperature conditions, and their effect on the effectiveness of DNA extraction and Short Tandem Repeat (STR) profiling are presented. Burned teeth followed the same macroscopic pattern previously described for bones, reaching a chalky white condition in 5 minutes at 400ºC; however, fractures were observed from 10 minutes at 300ºC, with differences between crowns and roots. DNA extraction was not possible from groups treated at 400ºC for 10 and 15 minutes; 500ºC for 15 minutes; 600ºC for 5 minutes; and 700ºC for 5, 10, and 15 minutes. For STR profiling, full amplification was possible after cremation, although it was very low after 1-5 minutes at 300ºC.

Last, an identification case example is presented in which DNA analysis could not distinguish between two brothers who died in the same car accident, but identification of each was possible through odontology.

Burned Human Remains, Burned Teeth, Forensic Odontology

G43 The Forensic Impact of the Humanitarian Work of the Vicente Ferrer Foundation's Rural Development Trust in India

Joe Adserias, DDS, PhD, C/ Balmes 62, Barcelona, SPAIN; Aida Dieguez, DDS, Galicia, Santiago, SPAIN; Sergio Irazusta, DDS, Barcelona, SPAIN; MD Y. Ballasubbaiah, MD, Rural Development Trust, Vicente Ferrer Foundation, Kanekal Cross, Kaneka, INDIA; and Vicente Lozano, PhD, University of Barcelona, C/ Feixa Llarga s/n, Hospitalet de llobregat, Barcelona 08907, SPAIN*

After attending this presentation, attendees will be aware of a new concept of humanitarian work in oral health, not only as a clinical issue but also as a source of antemortem dental records, that could be of great help in cases of identification.

This presentation will impact the forensic science community by describing the registration of medical history as a source of antemortem data in a place where the possibility of being identified without this information is very low.

In 1969, Vicente and Ann Ferrer founded Rural Development Trust (RDT), which is a non-governmental organization that implements welfare and comprehensive development programs in the region of Andhra Pradesh for marginalized and underprivileged communities, which primarily includes scheduled castes, scheduled tribes, backward castes, and people with disabilities. The area served by RDT is divided into seven regions: Bukkarayasaumdram, Bathalapalli, Kadiri, Srisailam, Uravakonda, Kalyandurg, and Madakasira. The organization's programs address major issues such as education, women's rights and progress, community healthcare, HIV/AIDS, hospitals, community habitat, community-based rehabilitation, and ecology. Sports and cultural programs are also organized because RDT believes that sports and cultural events are essential for the growth, self-esteem, and confidence of rural children. A team of approximately 2,400 people (99% are local individuals) manages the organization's development programs in education, health care, women, people with disabilities, housing, and ecology. It is one of the most innovative and sustainable Non-Governmental Organizations (NGOs) in India's history, providing coverage to more than 3,200 villages and nearly three million people. In 1996, the organization opened its first office in **Spain, The Vicente Ferrer Foundation,** to ensure stable funding and help sustain its projects in **India** with its headquarters in Barcelona and seven regional offices around Spain. In 2013, in a bid to raise funds in new markets and help ensure the continuity of projects in **India**, the organization opened its first United States office in Miami, FL. This delegation will promote awareness of, and raise funds for, the projects in India, so that the organization can continue to help thousands of families combat extreme poverty.

The goals of **RDT health programs** are **to ensure** that **the rural poor population has access to health care**, to raise awareness of healthy habits, and to ensure that affordable, quality health services are available through either the public system or the RDT-**Vicente Ferrer** institutional and community health care networks. Currently, there is one dental office at Kanekal Cross RDT Hospital, where two local odontologists, two local nurses, and volunteers who come for different periods of time are working. In 2014, Kanekal Cross Hospital Dental Office assisted 2,675 patients, provided 767 restorations, performed 271 root canal treatments, performed 889 extractions, provided 373 removable prostheses, and repaired 609 crowns. In the coming months, another dental office will be opened in Bathalapalli RDT Hospital. All RDT hospitals have a registered medical history and all medical and dental treatments are recorded. Medical histories of the patients are kept at the hospital to ensure that this information is secure. Panoramic dental radiography is limited to select cases at the current time, and the radiograph is retained by the patient. Medical history registered at RDT hospitals is the only antemortem data with biometric information that most of this Indian population has because some of them are not censused. Two years ago, the Indian government began to record and document fingerprints for national Identification (ID) cards.

In conclusion, the registration of treatments in RDT hospitals and specialty dental offices provides important antemortem data that can make the difference in whether or not an odontologist can perform identifications for criminal cases and mass disasters for the Andhra Pradesh Indian population.

Humanitarian Odontology, Disaster Victim Identification, Rural Development Trust

G44 Imaging Techniques for Intraoral Postmortem Dental Radiographs

Ann M. Bruhn, MS, Old Dominion University, 4608 Hampton Boulevard, Norfolk, VA 23529; Tara L. Newcomb, MS*, Old Dominion University SODH, 4608 Hampton Boulevard, Norfolk, VA 23529; and Bridget Giles, PhD, Old Dominion University, VMASC, 1030 University Boulevard, Suffolk, VA 23435*

After attending this presentation, attendees will better understand that dental hygienists are important active members of the forensic odontology team — specifically their ability to expose Postmortem (PM) intraoral dental radiographs for Antemortem (AM) comparison and identification using two different techniques. PM and AM intraoral radiographic image comparisons are among the most accurate and common methods of identifying human remains.[1-7] Imaging PM dental radiographs is part of the defined role of dental hygienists as mass fatality response team members, among others; however, this can be challenging when imaging fragmented, burned, and sheared dental remains.[4] While the paralleling and bisecting techniques are accepted practice for imaging PM periapical intraoral radiographic images, it is unknown which technique produces PM radiographs with fewer errors. This presentation presents a protocol for radiation safety and evidence-based practices for obtaining optimal dental forensic images utilizing dental hygienists as part of forensic radiography teams.

This presentation will impact the forensic science community by providing a training assessment tool and comparisons of two PM intraoral radiographic techniques. Attendees will also be updated on results of safety standards used in the study. Data presented will be beneficial to forensic odontologists, dental hygienists, and other bench-level forensic workers in staying informed about various techniques in the disciplines other than those in which they work.

Radiographers (N=38) obtained images using a hand-held dental X-ray system (NOMAD Pro™) with a Schick Elite digital sensor. Each radiographer obtained intraoral radiographic images of five fragments of fragile, broken, lubricated, real human skulls. Bisecting and paralleling techniques were used on the same five fragments for each skull, producing 10 images per radiographer, and generating a total of 380 images. All radiographic images were scored based on criteria adapted from Kieser et al. and using a Radiographic Evaluation Form (training assessment tool), which identified errors in the following categories: angulation, placement, exposure, and any other errors that did not fall within these categories.[8] Total errors and error scores were compared for statistical significance via one-tailed t-tests. The significance level was adjusted to α=0.01 (or α=0.05/5 comparisons).

It was hypothesized that the mean error for the bisecting technique would exceed the mean error for the paralleling technique, which was proven statistically. Mean total errors for the bisecting technique (M=12) exceeded mean errors for the paralleling technique (M=8) (p <0.001) with a large effect size of d=1. Participant dosimeter badges read a zero exposure count.

In conclusion, the results successfully differentiated between two different radiological techniques and proved that the paralleling technique had superior performance over the bisecting technique. The results also support participation of dental hygienists in initial and annual training for the proper protocols involved in obtaining high-quality PM intraoral radiographs. Further intraoral forensic radiographic imaging studies should include multidisciplinary teams (dentists, dental assistants, computer technicians, and dental hygienists) who are trained together for producing optimal PM images.

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Mass Fatality, Intraoral Radiography, Dental Hygiene

G45 Morphological Changes in Palatal Rugae After Maxillary Surgical Procedure: Is It Possible?

Antonio A. Antunes, PhD, Rua Cardeal Arcoverde, 267, Graças, Recife, Pernambuco, BRAZIL; Augusto P. Oliveira, University of Pernambuco, Faculty of Dentistry, Av. General Newton Cavalcanti, s/n, Camaragibe, BRAZIL; Evelyne P. Soriano, PhD, Faculty of Dentistry, University of Pernambuco, Av. General Newton Cavalcanti, s/n, Camaragibe, BRAZIL; Marcus Vitor D. Carvalho, PhD, Faculty of Dentistry, University of Pernambuco, Av. General Newton Cavalcanti, s/n, Camaragibe, BRAZIL; Reginaldo I.C. Campello, PhD, Faculty of Dentistry, University of Pernambuco, Av. General Newton Cavalcanti, s/n, Camaragibe, BRAZIL; and Gabriela G. Porto, PhD, Faculty of Dentistry, University of Pernambuco, Av. General Newton Cavalcanti, s/n, Camaragibe, BRAZIL*

After attending this presentation, attendees will better understand morphological pattern changes suffered by the palatal rugae in patients who experienced surgically assisted maxillary expansion. Attendees will be able to compare morphological patterns before and after the surgical procedure.

This presentation will impact the forensic science community by providing results on the real impact of this surgical procedure in the palatal rugae morphology. This data will optimize the human identification process when this technique is employed.

Human identification is an important step in civil and criminal cases. Dental characteristics, dermatoglyphics, and DNA comparisons are commonly and systematically used techniques for rapid and secure human identification; however, some of these techniques may not be available due to specific crime scene conditions or the absence of experienced staff. An increasing interest in alternative and reliable methods of human identification can be observed in the literature.[1-3] One of these alternative techniques is cheiloscopy, which involves cranial measurements in the mastoid process of occipital bone, clavicle, humerus, radius, and ulna.[4,5] Another technique is palatal rugoscopy since the palatal rugae are highly variable yet specifically individualized and stable over time.[4] The palatal rugae have been considered as an useful identification marker, even in radically compromised bodies.[3] Previous studies report that there are no significant changes in palatal rugae in 93% of burnt bodies and in 77% of bodies seven days after death.[6] Surgically assisted maxillary expansion is indicated for cases of maxillary transversal growth deficiency. An orthodontic surgical therapy is instituted to correct this deficiency in order to increase transversal dimensions with the aid of an orthodontic device.[7] Although palatal rugoscopy occupies a prominent place in human identification, there is still no scientific evidence regarding palatal rugae morphological changes when this surgical procedure is performed, nor how this procedure can influence and alter the effectiveness and reliability of palatal rugoscopy.

To address these questions, a prospective controlled study was developed. Regular patients at the Faculty of Dentistry, University of Pernambuco Oral and Maxillofacial Surgery service who requested surgically assisted maxillary expansion treatment were selected for this study. Study models were obtained in the pretreatment period and at the time of treatment completion. The cohort samples were divided into three groups: (1) Group A — dental casts were taken of patients in the period before the surgical procedure; (2) Group B — dental casts of the same patients were obtained after the maxillary expansion procedure; and, (3) Group C — dental casts were acquired of randomly selected normal patients that did not need treatment for maxillary surgical expansion (control group). Rugae morphological classifications developed by Thomas and Kotze and Hauser et al. were used in this study, which defined the morphological patterns of the primary rugae, incisive papillae, direction of rugae alignment, and midpalatal raphe morphology.[8,9] A second classification system developed by Santos was also adopted for this study.[10] Results were evaluated with descriptive statistics such as mean, standard deviation, and percentage; for comparison analyses, chi-square and Wilcoxon tests were used.

The sample included 16 patients in Group A (pre-treatment), 16 patients in Group B (post-treatment), and 16 patients in Group C (untreated control). In 81.25% of these cases, there were changes in primary rugae morphologies in comparison to Groups A and B (p=0392). The branched form was the most prevalent in both sides (31.25%) of both Groups A and B. Incisive papillae shape evaluation identified changes in 68.75% of the cases when comparing Groups A and B (p=0.001). The most prevalent shape was the diamond (37.5%), followed by the minimum, with 31.25% for both groups. Rugae alignment direction was altered in 56.25% of the cases when comparing Groups A and B (p=0.002). The horizontal alignment was the most common and was observed in 56.25% of the cases. Median palatal raphe morphology was not coincident in 56.25% of the cases in A and B group comparisons (p=0.432). The straight shape was the most frequent (43.75%), followed by waved (37.5%).

This study determined that palatal rugae morphology can be altered after surgically assisted maxillary expansion, which can interfere with the identification process. Dental records should be carefully evaluated for notations of this procedure before utilizing palatal rugoscopy as a forensic method for human identification.

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Palatal Rugoscopy, Human Identification, Orthognathic Surgery

G46 The Computer Program for Identification of the International Criminal Police Organization (INTERPOL) Disaster Victim Identification (DVI) System International (Plass Data System) — The New Web-Based Version 5: Changes and Discussion

Tore T. Solheim, Box 1052 Blindern, Inst Moral Biol, Oslo 0316, NORWAY*

After attending this presentation, attendees will better understand the construction of the program, DVI System International, new version 5. Detailed knowledge of the dental forms 600 will be acquired and some critical comments will be presented. Attendees will also be aware of the new shortened set of abbreviations of the dental features.

This presentation will impact the forensic science community by enabling attendees to more easily learn how to use the program and thus be able to participate in international DVI operations.

DVI System International was introduced after 1990 as a Disk Operating System (DOS) version. It was subsequently updated in version 3 to a Windows® program, and version 3 was used after the 2004 Asian tsunami. A number of changes have been made to the program and to the INTERPOL DVI forms that are the basis of the program. The new web-based version 5 was introduced on October 21, 2014. The new version contains numerous changes, both in the layout of the forms and in the program functionality. The new version has been so extensively upgraded that it is almost a totally new program. Working knowledge of version 3 will not be sufficient for fluency in version 5. Without proper training, some users may be unable to work with version 5.

Version 5 contains two authorization stages for odontologists. Only authorized dental administrators are allowed to generate final comparison reports. Normal, unauthorized users (designated as "editor") can enter Antemortem (AM) and Postmortem (PM) data and perform comparisons, but the results will only indicate a possible identity. Another user level (designated as "reader") has an even lower authorization level; these persons can only read what is entered in the databases, but they cannot enter or change data. This authorization level would not be applicable for odontologists working in a real disaster.

Data entry is not difficult, but the number of abbreviations of dental information has been reduced. This should not introduce problems because data can still be entered using quotation marks; however, there are some problems in entering data on the dental surfaces. These operations are more difficult in version 5, and the procedures will be explained in this presentation. The generation of comparisons and comparison reports are much more difficult than before due to extensive changes in the protocols. The three authorization levels for users include greater controls and safety mechanisms so that the conclusions that are made are really authorized by responsible dentists. Some think that is unnecessary, especially because it complicates comparisons. There are many steps in the process and users can easily go astray. Without detailed knowledge of each step, one is unable to generate a comparison. As in version 3, users can search "all against all" or one AM/PM case against all PM/AM cases. Users can also search for a certain type of treatment or combination of treatments. Critical comments will be discussed for the new version 5 program.

DVI, Computer Program, INTERPOL

G47 New Mexico Office of the Medical Investigator: Overview, Dental Identification Statistical Data, and Mass Fatality Incident Plan

Cristina M. Dalle Grave, DDS, 380 Via Del Rey, Angel Fire, NM 87710; and Peter W. Loomis, DDS, NM Office of the Medical Investigator, 700 Ranchitos Road, NW, Los Ranchos, NM 87114*

After attending this presentation, attendees will: (1) be familiar with the Office of the Medical Investigator in New Mexico; (2) be shown a comparison of the statistical data of the dental identification cases in the state of New Mexico; and, (3) understand the dental identification aspect of the New Mexico Mass Fatality Incident Plan with an example of a small mass fatality that happened in 2014.

This presentation will impact the forensic science community by providing an increased understanding of the New Mexico dental forensic system and data, and the importance of having a mass fatality plan in place.

Medical examiner reports are an important and rich source of data for epidemiological research. The New Mexico Office of the Medical Investigator (OMI) was created by the New Mexico State Legislature in 1972 and became operational in 1973. The OMI replaced the county coroner system, and is the statewide, centralized medical examiner agency for investigating any death occurring in New Mexico that is sudden, violent, untimely, unexpected, or when a person is found dead and the cause of death is unknown. Each year, the OMI investigates approximately one-third of all deaths in New Mexico (2014 state population of 2,085,572), and all scientific procedures are performed at a centralized state-of-the-art forensic facility in Albuquerque, NM. The scientific services of the OMI include medicolegal death investigation, forensic autopsy, hospital and consult autopsy, expert witness testimony, forensic pathology consultation, forensic anthropology, forensic epidemiology, forensic odontology, and forensic radiology. It is the only medical examiner in the United States equipped with X-ray, Computed Tomography (CT), and Magnetic Resonance Imaging (MRI) scanners. The OMI also provides consulting services for requesting agencies since it does not have jurisdiction over certain federal areas in the state (military and tribal). The OMI has maintained electronic records of death investigations, autopsies, and dental forensic data since 1975; frequently, this information is very useful for epidemiological studies. The OMI is designated as a special program within the Department of Pathology at the University of New Mexico (UNM), School of Medicine; the program operates out of the OMI Central Office located near the UNM Health Sciences Center in Albuquerque.

In 2013, New Mexico had 5,577 deaths that met the criteria to become a reportable death. The OMI provided investigative services for each of these deaths. In 2014, the forensic odontology service completed 159 postmortem dental examinations on unidentified persons. Each year, the OMI processes 150 to 200 cases in which the remains are initially unidentified; 98% of those cases are eventually successfully identified through fingerprint, dental, anthropologic, radiographic, or DNA comparisons.

The forensic odontology service of the OMI uses WinID and DEXIS™ software. The WinID software stores data in a Microsoft® Access® database, which is helpful for statistical data studies. The WinID OMI database contains more than 1,200 postmortem entries from 2006 to 2015. Using this database, it is possible to analyze and compare the following parameters: year that the body was found, gender, race, postmortem condition, and dental information. There are still 89 active postmortem cases out of 1,200 postmortem dental records, reflecting the importance of odontological methods as a valid and reliable personal identification procedure. The statistical data of the 1,200 cases will be discussed during the presentation.

After several mass fatality incidents including the Oklahoma City bombing, the September 11 terrorist attacks, Hurricane Katrina, and the Joplin, MO, tornado, the use of dental records for human identification has become increasingly important. The 2012 New Mexico Mass Fatality Incident Plan provides detailed protocols and Standard Operating Procedues (SOPs) for processing and examination of postmortem dental evidence and comparing this evidence with antemortem dental and medical records.

In this presentation, a mass fatality incident will be discussed that occurred on 05/26/2014 in New Mexico and included 13 victims, 11 of whom were burned and two of whom were in an advanced stage of decomposition. This event will be presented to validate the necessity of having a mass fatality incident plan in place.

Forensic Odontology, Dental Identification, Mass Fatality Incident

G48 The Use of Dental Patterns in Decedent Identification: The Role of the New and Improved OdontoSearch 3.0 Program

Kenneth W. Aschheim, DDS, 44 E 67th Street, New York, NY 10065; and Bradley J. Adams, PhD*, New York OCME, 520 1st Avenue, New York, NY 10016*

After attending this presentation, attendees will be informed regarding the use of the OdontoSearch 3.0 program in dental identification. Instructions on the use of the program will be provided as well as information about the reference data. Case examples will be used to highlight how the OdontoSearch program can be used in the field of forensic odontology. The OdontoSearch 3.0 program provides an easy-to-use interface and customizable searches that will assist forensic odontologists in their casework

This presentation will impact the forensic science community by introducing a new version of the OdontoSearch program. In addition, this presentation will demonstrate how to utilize the frequency of occurrence for dental restorations in combination with concurring contextual evidence to identify a decadent in the absence of antemortem radiographs.

Dental identification via comparison of antemortem and postmortem X-rays is a well-accepted and reliable method of decedent identification. In some cases, antemortem dental X-rays may not be available, but treatment records may contain notes and diagrams that can still be used for comparison. The problem with dental treatment charts and notes is that, unlike X-rays, the information cannot be shown to be exclusively correlated with a specific individual (i.e., numerous people may have the same teeth filled or extracted). In the past, the strength of a match between a missing person's dental treatment records and the treatments observed on an unidentified set of remains was based on the clinical experience of the dentist. This interpretation is subjective because different dentists may arrive at very different conclusions even though they are considering the same records.

The original OdontoSearch computer program was developed to provide an objective means of assessing the frequency of occurrence for dental treatment.[1] OdontoSearch 3.0 is an update of this online program that provides immediate frequency results and adds additional features. The statistical values provided by OdontoSearch provide forensic odontologists with an objective means of quantifying the relative frequency with which a sequence of dental characteristics would be expected to occur in the general population. The program works by coding the condition of the individual teeth (missing, filled, or unrestored) using a graphical odontogram interface to create an overall dental pattern (i.e., the dental pattern is created by forming a sequence with the tooth codes). The resulting pattern is then compared to a large, representative sample of the United States population to determine the pattern frequency within the population. The methodology and rationale behind the OdontoSearch program is very similar to the statistical procedures that have been established for mitochondrial DNA profile comparisons. Use of the original OdontoSearch program confirmed that there was a large amount of diversity in the dental pattern sequences created by missing, filled, and unrestored teeth, and the updated program has expanded the database size by more than 50%.[2]

With the OdontoSearch 3.0 program, uncommon dental patterns can be recognized as such, and a frequency value can be associated with specific patterns. In many instances, these results may be counterintuitive because the presence of only a few common fillings may still create a very rare dental pattern when all of the teeth are considered as a whole sequence. The OdontoSearch results may be used along with other analytical information (e.g., skeletal analysis, personal effects, and geographic area) to build a convincing case for identification of a specific individual.

The new version of OdontoSearch (version 3.0) is now available at a new web address (www.odontosearch.com). OdontoSearch 3.0 includes numerous improvements and updates from earlier versions of the program. These include: (1) an improved user-interface that presents an odontogram and allows for easier data entry; (2) new reference data from the National Health and Nutrition Examination Survey (NHANES); (3) expansion of the age parameters in the reference data to include individuals between the ages of 14 years and 90 years old; (4) a reference dataset of 57,980 individuals; and, (5) customizable searches so the user can select different parameters for age, sex, and ancestry. For example, if the user was only interested in observing the frequency of a specific dental pattern in Caucasian males between 30 years and 60 years of age, the OdontoSearch 3.0 program would be able to provide these results.

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OdontoSearch, Forensic Odontology, Dental Identification

G49 Dental Encoding Translator Applications Suite (DEnTAS) — Universal Dental Code Translator

Kenneth W. Aschheim, DDS, 44 E 67th Street, New York, NY 10065; and Bruce Bandini, MS*, NIST, 100 Bureau Drive, Gaithersburg, MD 20899*

After attending this presentation, attendees will better understand the DEnTAS 1.1.0 program.

This presentation will impact the forensic science community by being the first step in allowing for the universal translation of dental codes between major dental forensic identification software packages utilizing the American National Standards Institute/ National Institute of Standards and Technology-Information Technology Laboratory (ANSI/NIST-ITL) biometric data exchange's Extensible Markup Language (XML).

DEnTAS 1.1.0 is the first version of the software that supports translation and data exchange of dental codes used by major dental forensic identification software products. Based on the ANSI/NIST-ITL 2013 Dental Supplement, it creates an intermediary Type 12 (forensic dental and oral data) and Type 22 (imagery) XML file that can easily translate the dental codes. Case examples will be used to highlight how: (1) the DEnTAS program acquires native codes from major dental forensic identification softwares such as WinID and U-Dimension (UDIM); (2) the source dental codes from the identification software are translated into an intermediary XML file; and, (3) the intermediary XML file codes are exported into all the supported destination formats.[1-3]

Dental data has a long history as a primary identification modality. Unfortunately, the lack of standardization in the coding between forensic dental identification software packages and practice management software makes the electronic transfer of these data virtually impossible. The inclusion of the forensic dental data into the 2013 Dental Supplement ANSI/NIST-ITL Forensic Standard and the adoption of the National Information Exchange Model (NIEM) encoding for biometrics data is the foundation for the intra-operability between software packages. Currently, exchange of this type of data: (1) is prone to error and subjective interpretation; (2) is time consuming; and, (3) requires manual translation of dental codes by users of different dental programs.

DEnTAS 1.1.0 is the first version of a free, publicly available software package that will allow the translation of this information. The basic program consists of an input and output section and a "translation" engine. The lynchpin of the DEnTAS program is the dental code translation table. This editable table translates the Forensic Identification Software or Practice Management Software data into an intermediate ANSI/NIST-ITL file (Type 12 and Type 22 data) as an XML-compliant file. The presence of multiple translation tables allows the DEnTAS software to input one set of data, translate it into the ANSI/NIST-ITL file, then take that output file and retranslate it into a second software package of forensic dental data.

An example would be a situation in which dental data was originally recorded using UDIM, and a WinID User wants to import these data. Prior to the DEnTAS software, a new record in the WinID program first had to be manually created. Then, the dental codes from UDIM had to be translated into the WinID code set and manually entered into WinID. The DEnTAS program removes the translation and manual entry steps in the data exchange process. DEnTAS automates the entry and translation process and supports a subset of the dental codes and their corresponding (software) programs.[4]

The final goal of this project is to create numerous translational tables for an infinite number of translations. Ultimately, the goal would be that the Odontology Subcommittee of the Crime Scene/Death Investigation Scientific Area Committee (SAC), under the Forensic Science Standards Board (FSSB), would be responsible for the maintenance and certification of these tables.

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2. ANSI/ADA Standard No. 1058 for Forensic Dental Data Set.
3. www.niem.gov.
4. Table 86, Dental System Codes of NIST SP 500-290 Rev 1. 2013.

Forensic Odontology, Dental Codes Translation, UDIM

G50 The Odontologist's Role in Death Investigation in Cases of Deaths After Dentistry

Yolanda Nerkowski, BA, Ontario Forensic Pathology Service, 25 Morton Shulman Avenue, Toronto, ON M3M 0B1, CANADA; Taylor L. Gardner, BFSc, Ontario Forensic Pathology Service, 25 Morton Shulman Avenue, Toronto, ON M3M 0B1, CANADA; Jeff Chadwick, DDS, Princess Margaret Hospital, 610 University Avenue, Rm 2-933, Toronto, ON M5G2M9, CANADA; Kris Cunningham, MD, Ontario Forensic Pathology Service, 25 Morton Shulman Drive, Toronto, ON M3M0B1, CANADA; and Robert E. Wood, DDS, PhD, Princess Margaret Hospital, 610 University Avenue, Toronto, ON M5G 2M9, CANADA*

After attending this presentation, attendees will understand the role of the expert forensic dentist in assisting the death investigation team in examination of means, manner, and cause of death in cases in which death has occurred close to the time of dental procedures.

Deaths in the course of dental treatment are rare; however, from time to time, the odontologist, as a practicing dentist and a member of the death investigation team, will be called upon to provide an opinion as to the appropriateness of care prior to, during, and following a patient's demise. This presentation will impact the forensic science community by highlighting input of the odontologist who, in these cases, assists the forensic pathologist in establishing the means and manner of death.

> "There are known knowns – things we know we know. There are some things we do not know. And also unknown unknowns – the ones we don't know we don't know."
>
> Donald Rumsfeld

Some people die as a consequence of "known knowns." In one review of 5,000 orthognathic surgery patients, there were 20 fatalities. The causes were multifactorial. One young man died days after the surgery. The forensic odontologist attended the autopsy to assess radiographic images and clinical aspects of the autopsy as it applied to the particular orthognathic procedure, in an attempt to assess whether anything was amiss. The procedure was competently performed, fixation plates were in place, and there did not appear to be any oral surgical issues. Autopsy revealed parapharyngeal hemorrhage leading to death. This case demonstrates that when death is even remotely possible after a given procedure, heightened patient monitoring is justified.

Death also may occur as a consequence of mismanaged dental infection, so-called "known unknowns." Dental infections can resolve with minimal clinical care, or they can lead to serious, rapidly progressing, even fatal consequences. A 19-year-old male visited his dentist with jaw pain and swelling. The dentist placed him on antibiotics. The young man ultimately went to an Emergency Room (ER) at a major urban hospital. What began as a simple dental infection rapidly progressed to Ludwig's angina. The admitting diagnosis in the ER was Ludwig's angina, and this was the stated cause of death. The main issue facing the forensic odontologist was that no additional treatment was rendered as the disease progressed and, when the patient developed severe air hunger, a "violent patient code" was called instead of a medical emergency code. The patient went Vital Signs Absent (VSA), and despite attendance by the cardiac arrest team and aggressive Cardiopulmonary Resuscitation (CPR) efforts, the man died. The forensic odontologist in this case was contacted by the coroner to review all pertinent records as part of the coroner's investigation. Issues were identified involving staffing, triage, providing treatment that matched the diagnosis, and substandard care by some staff and the institution.

Then there are cases of "unknown unknowns." A woman had visited the dentist on numerous occasions during several months for root canal treatment and major restorative reconstructive dental care. Her final appointment was for a simple crown cementation and the procedure was uneventful. The woman was discharged by the dentist but became unwell, experiencing burning pain in the center of her back while in the waiting room. She never lost her pulse or breathing. The dentist called 911, placed the patient in the dental chair, administered oxygen, and stayed with the patient until Emergency Medical Services (EMS) arrived. She died a few days later. The forensic odontologist was called to review the nature of the dental work. The family was concerned that the patient was receiving "implants" before her death, and believed that the stress of this surgical procedure led to her death. The forensic odontologist was asked by the forensic pathologist to provide context regarding the relative stresses of the dental procedure. The autopsy showed that the woman died of a dissecting aortic aneurysm, which could have occurred at any time and in any place.

Dentistry, Death, Odontology

G51 How to Deliver Sub-Optimal Dental Care Effectively

Taylor L. Gardner, BFSc, Ontario Forensic Pathology Service, 25 Morton Shulman Avenue, Toronto, ON M3M 0B1, CANADA; Yolanda Nerkowski, BA, Ontario Forensic Pathology Service, 25 Morton Shulman Avenue, Toronto, ON M3M 0B1, CANADA; Jeff Chadwick, DDS, Princess Margaret Hospital, 610 University Avenue, Rm 2-933, Toronto, ON M5G2M9, CANADA; Kris Cunningham, MD, Ontario Forensic Pathology Service, 25 Morton Shulman Drive, Toronto, ON M3M0B1, CANADA; and Robert E. Wood, DDS, PhD, Princess Margaret Hospital, 610 University Avenue, Toronto, ON M5G 2M9, CANADA*

After attending this presentation, attendees will be aware that in some critically-ill patients that require emergent dental care, decision trees have to be modified to suit the situational parameters.

This presentation will impact the forensic science community by increasing awareness of how common compromised care is in North America. Approximately one-half of patients do not see a dentist. Populations in North America are aging and these patients are attending acute-care hospitals for treatments such as heart valve surgery, cardiac assist devices, immunosuppressive chemotherapy, and multi-organ transplants. If half of the patients do not have regular dental care, then many of these critically ill patients, and the attending professionals, will face complex and medically risky decisions at critical junctures in their medical management.

The American Society of Anesthesiologists (ASA) patient risk and severity classification extends from "I" to "V," with the former being healthy and the latter unlikely to survive 24 hours. This presentation discusses four patients in the ASA IV and V classes that required urgent and immediate ameliorative dental care while they were in medical crises.

The first patient, a 23-year-old female, had anti-NMDA receptor encephalopathy, was unconscious, had a tracheostomy, was ventilated, had epileptiform seizures, had bitten the end of her tongue off, avulsed all or parts of three teeth, dislodged an orthodontic wire into the soft tissues of her face, and had lacerations to the inside of her mouth. She also was incapable of giving consent. With the consent of her parents, one tooth and parts of another tooth were removed in the neurology Intensive Care Unit (ICU), and her jaws were wired together to prevent further laceration. This procedure was performed to prevent the possibility of aspiration and reduce the risk of further soft tissue injury. The procedure was performed two more times on later occasions.

A second patient, a 49-year-old male with non-ischemic cardiomyopathy awaiting urgent placement on a Left-Ventricular Assist Device (LVAD), had not seen a dentist in 25 years. He could not be moved from Cardiac ICU (CICU) and required inotropic support. Approximately ten clinically infected teeth were removed and hemostasis was gained in CICU without transporting the patient to dentistry. Non-infected carious teeth were not removed. Removal of sites of infective foci is imperative in heart transplant patients receiving the bridging technology of LVAD.

A third patient, a 48-year-old male, had end-stage coronary artery disease with one coronary vessel having 100% blockage and another having 80% blockage. His coronary surgery was canceled while he was lying on the operating table by an observant anesthesiologist who observed that the man had a suppurating dental infection. The patient required extraction of seven infected teeth in the cardiac care unit to prevent endocarditis and to remove this budding space infection.

A fourth patient, a 67-year-old ventilated but awake female lung transplant patient, was seen one day after lung transplant. Her front teeth had been avulsed by an anesthetist, but "some" roots were left inside her jaw. She was in transplant ICU. Her husband had her dental chart, X-rays, and the avulsed bridge for inspection. With the aid of her dental records, further treatment was avoided until she became well enough to attend the dental clinic.

Critical elements of these cases include: (1) the emergent nature of the situation and recognition that "doing nothing" is doing something, and is not an option; (2) the importance of gaining consent from the patient or next-of-kin; (3) using the minimal amount of sedation/anesthesia and performing the procedure with close medical monitoring as near to the patient unit as is feasible; (4) the immediate goal of treatment in these individuals is, by definition, suboptimal — removal of infection and prevention of harm is the goal; and, (5) preparing the patient for definitive treatment in the appropriate dental clinic when their medical condition improves and progresses to lower levels on the ASA scale.

In the decision tree for these patients, the principles of non-maleficence (the duty to not cause or increase the risk of pain and suffering, incapacitation, or deprive others of life) and beneficence (the need to contribute to the critically ill patient's welfare) were maintained in all four cases. Evaluation of the clinical and legal aspects of these cases revealed that they fulfilled the principles of non-maleficence and beneficence, even though the care was sub-optimal.

Dentistry, Medically-Compromised, Decision Trees

G52 Dental Litigation Epidemiology

Francesco Pradella, MSc, University of Firenze, Dept of Forensic Medical Sciences, L.go Brambilla, 3, Firenze 50134, ITALY; Vilma Pinchi, PhD, via Della Resistenza 14, Murlo, Siena 53016, ITALY; Stefano Garatti, MSc, Azienda Desio E Vimercate, Via Bianchi, Carate Brianza, ITALY; Viola Bartolini, Largo Brambilla 3, Firenze, ITALY; and Martina Focardi, Largo Brambilla 3, Florence 50134, ITALY; and Gian A. Norelli, sez.dep.Medicina Legale, Firenze, ITALY*

After attending this presentation, attendees will better understand the phenomenon of dental litigation. Even though the data comes from the Italian context, it may be considered of worldwide value and interesting to understand the current trends in the issue.

This presentation will impact the forensic science community by providing results from a scientific and statistical analysis of the phenomenon of dental malpractice and litigation, especially useful because very little literature, written with scientific criteria, is currently available.

Goal: According to the current general opinion, dental litigations are increasing. The goal of this research is to provide a scientific representation of the dimensions of dental litigation and to analyze the most important features of the litigations (incidence by discipline, compensations) in private practice, public service, judicial, and extra-judicial contexts.

Materials and Methods: Data related to dental litigations have been collected from three primary sources: (1) private practice and insurance companies — by collecting data from the Italian National Dental Association (ANDI) and related to the professional liability policy specifically dedicated to members between 2002 and 2012 (12,000 subscriptions in 2012); (2) dental public service — by analyzing data for 2008-2012 from the Firenze University Hospital and AO of Desio and Vimercate, which provides 250,000 dental treatments per year in an area inhabited by 750,000 people; and, (3) the judiciary system — by analyzing the 2007-2013 rulings of the civil section of the Magistrates' Court of the city of Rome.

Results: The number of disputes in the insurance context (related to private practice) was 2.6% in 2002 and 4.6% in 2012. In the public service sector, the incidence of litigations was 0.16% in 2008 and 0.40% in 2012, with a higher rate of specious disputes in 2012. During 2007-2012, court settlements related to dental malpractice declined to 4% compared to 9.5% during 2001-2008. The plaintiff/dentist lost in 80% of the cases in the judiciary and in 95% of the cases with insurance companies. Prosthodontics and implantology are the dental specialties most affected by litigation. The breach of duty of informed consent was the cause of litigation in less than 3% of the cases. In comparison with the average sum compensated in the insurance context (from 11,000 EUR in 2002 to 4,700 EUR in 2012), the amounts awarded by the Magistrates' Court of Rome (>20,000 EUR) are much higher.

Conclusions: Dental litigation has a very low incidence ratio, between 1:500 and 1:1,250 of all dental patients. A technical error is the main cause of litigation, whereas breach of information is the cause of <3% of litigations. The dentist loses in 80%-95% of litigation cases, primarily because the dentist has the burden of proof in civil proceedings and even a simple lack of documentation can lead to an unfavorable outcome. In one-third of court trials, dentists have no insurance policy. The compensated sums appear, on average, larger at the outcome of a judicial procedure (mean >20,000 EUR) than after an out-of-court management of the dispute. Therefore, when the professional error is evident, persistence in the dispute generally involves higher expenses and significant prolongation of time and emotional stress for all parties. The results of the present research indicate that litigations in private practice and public service are not increasing in the judicial or out-of-court/insurance context, in the number of cases, or in the amount of compensation.

Professional Liability, Negligence, Litigation

G53 The Evaluation of 73 Dental Malpractice Cases From the Counsel of Forensic Medicine

Huseyin Afsin, PhD, Hacikadin Caddesi 18-1, Kocamustafapasa, Istanbul 3400, TURKEY; Ahmet Sadi Cagdir, MD, Council of Forensic Medicine, Adli Tip Kurumu Baskanligi Kimiz Sok No1, Cobancesme Bahcelievler, Istanbul, TURKEY; Abdi Ozaslan, MD, Istanbul University, Cerrahpasa Medical Faculty, Forensic Medicine Dept, Fatih, Istanbul 34098, TURKEY; Muhammet Nabi Kantarci, Council of Forensic Medicine, Kimiz sk. Çobançesme Mh, Bahçelievler, Istanbul, TURKEY; Umit Naci Gundogmus, The Council of Forensic Medicine, Ministry of Justice, Istanbul, TURKEY; and Gulnaz T. Javan, PhD, Alabama State University, Forensic Science Program, 915 S Jackson Street, Montgomery, AL 36104*

After attending this presentation, attendees will be familiar with types of malpractice lawsuits filed against dentists in Turkey.

This presentation will impact the forensic science community by describing the distribution of malpractice lawsuits in Turkey according to different disciplines of dentistry.

Harm caused because of substandard care is the reason for most dental malpractice cases. Like other malpractice cases, the numbers of dental malpractice cases are increasing, especially after changes that were made to Turkey's compensation laws in 2005. It is important that judgment on these malpractice cases be fair and just.

Dental malpractice claims are encountered as fines and lawsuits. According to the legal system of the Republic of Turkey, dental malpractice cases can be settled by fines, monetary punishments, or both. A proven malpractice case will result in a monetary fine and/or imprisonment. It also is possible that the practitioner could face punishment by the appropriate professional board. Expert witnesses are used in Turkish court systems to evaluate malpractice cases and can be from forensic medicine, dentistry, or faculty members. Dentists can be hired as an expert witness. The most preferred experts are members of forensic medicine. There is a department for evaluating malpractice cases within the forensic science department.

This study examined 73 cases of malpractice filed at the Institute of Forensic Medicine. Of these, 49.3% were referred by the prosecutor's office for investigation. A total of 60% (n=44) of the complaints were filed by males and 49% (n=29) were filed by females. The average age of male and female complainants was 31.55 years and 37.45 years, respectively. A total of 76.7% (n=56) of the claimants were treated at a private practice site, 12.3% (n=9) at family health centers, 6.8% (n=5) at state hospitals, and 4.1% (n=3) at teaching hospitals. Distributions of practitioners according to their education levels were as follows: 89% (n=65) were general dentists, 9.6% (n=7) were specialist dentists, and 1.4% (n=1) were dental technicians. Most lawsuits were filed after a tooth extraction; 14 of these cases were related to wisdom tooth extraction and 12 involved extraction of other teeth. There were 13 cases filed for prosthetic crown procedures, 12 cases for implants, and one case for falsifying a medical report.

The majority of malpractice cases (76.7%) were filed against private practices and the least number of complaints (4.1%) were filed against teaching hospitals. Of the total complaints against dentists, 89% were filed against general practitioners. Evaluation of the 73 malpractice cases filed against dentists in the forensic medicine institute's files revealed that 24 cases (33%) had merit and 49 cases (67%) were frivolous lawsuits. There was no statistically significance difference (p>0.05) with respect to gender in the studied cases.

Like any other medical practice lawsuits, protection of patients' rights and appropriate care should be an important part of the outcome of dental malpractice. Patient-doctor relationships, the attitude of the staff toward patients, empathy, and patients' sense of confidence play significant roles in reducing malpractice complaints against practitioners. Educating doctors and following the standards of practice by practitioners have proven to reduce the numbers of lawsuits filed against dentists.

Dental malpractice, Council of Forensic Medicine, Turkey

G54 Partial Faceoff Dissection in Dental Autopsy

William E. Silver, DDS, 10 Edgewater Drive, #5G, Coral Gables, FL 33133; and Richard R. Souviron, DDS, 336 Alhambra Circle, Coral Gables, FL 33134*

After attending this presentation, attendees will recognize and facilitate indications for partial facial dissection to preserve identity.

This presentation will impact the forensic science community by demonstrating how examination and reconstitution of dental structures during the course of a dental autopsy may be accomplished without facial disfigurement.

Forensic examination of the teeth and jaws is difficult without adequate exposure. The partial faceoff dissection procedure, which is enhanced by proper jaw removal, permits adequate examination of the mandible and maxilla and enables the replacement of any disturbed anatomical elements to their original visual condition. Forensic photography and radiography are additional benefits of this procedure. The forensic odontologist is often presented with a difficult means of access to the teeth and jaws due to the size or condition of the soft tissues surrounding the oral cavity. Adequate exposure of the teeth is essential for proper charting, photography, and radiography of the teeth during the dental autopsy. During the course of the medical autopsy, it may be advisable to reflect the entire facial soft tissue integument in order to discover previously unknown locations of underlying trauma and still preserve the facial appearance.

Full face removal is not essential for the dental autopsy. This is described in detail by diagrams and during the autopsy. The partial faceoff is a derivative of the full faceoff. This is represented by two actual cases at the time of dental autopsy, and may require the prior approval of the chief medical examiner so as not to interfere with the medical autopsy forensic examination. An initial scalpel cut is initiated on a level just below the zygomatic arch, anterior to the right ear, on a line descendent from the outer canthus of the eye. It proceeds across the cheek to a line inferior to the nares in order to expose the floor of the nasal cavity, and continues to the same area on the left side as on the right. This continuous cut then turns downward toward the inferior border of the mandible, follows a line just above the inferior border of the mandible back to the opposite side, and terminates before cutting superiorly, thus leaving a hinged area on the right side that is the "viewing side."

In cases involving the need to examine the teeth and oral cavity when traditional dissection is not advisable because of the necessity to maintain visual access and preservation of tissue, this dissection may be performed as an alternative to the standard forensic odontological examination to prevent damage or mutilation of the face while still exposing the teeth and oral cavity. Traumatic damage to the teeth and jaws would be revealed before opening the jaws during this procedure. When rigor is still in place, this allows the operator to access the dental apparatus and secure a better opening of the jaws for examination. For further access, it is possible to remove the maxilla and/or mandible for a separate examination outside of the body and to obtain better visualization of all the teeth. Upon completion of the forensic examination, the maxilla and mandible may be returned to their former anatomical positions in order to secure a more complete restoration of the original facial configuration.

Finally, replacement of the dissected portion of tissue is accomplished by returning the hinged flap to its original position and closely approximating the tissue edges. Stabilizing the tissue can be achieved by fine suturing the edges or by applying cyanoacrylate glue along the edges of the incision.

Faceoff, Autopsy, Dental

G55 3D Analysis of Dental Crown Morphology in Laser-Scanned Dentitions: A Comparison of Three Software Packages

Ademir Franco, MSc, Katholieke Universiteit Leuven, Kapucijnenvoer 7, block a, Leuven, BELGIUM; Guy Willems, PhD, Katholieke Universiteit Leuven, School of Dentistry, Kapucijnenvoer 7, Leuven B-3000, BELGIUM; Sérgio Ignácio, PhD, Pontifícia Universidade Católica do Paraná, R. Imac. Conceição, 1155, Curitiba PR80215-901, BRAZIL; Paulo Souza, PhD, PUCPR, R. Imac. Conceição, 1155, Curitiba PR, 80215-901, BRAZIL; and Patrick W. Thevissen, PhD, KULeuven, Dendermondsesteenweg 483, Sint-Amandsberg, Oost Vlaanderen B-9040, BELGIUM*

After attending this presentation, attendees will: (1) be updated regarding the current investigations on the uniqueness of the human dentition; (2) understand the relevance of 3D morphological analysis of dental crowns in the context of forensic sciences; and, (3) be aware of an ideal software set-up to investigate the uniqueness of dental crowns.

This presentation will impact the forensic science community by exposing the current limitations on the investigation of the uniqueness of the human dentition. Moreover, the performances of three existing 3D software packages used to analyze metrics and superimpositions of the dental crowns will be evaluated and compared.

The existence or lack of uniqueness in human dentition became one of the most polemic topics in forensic sciences in recent years.[1] In 2009, the National Academy of Sciences highlighted the social impact of this controversy, exposing the harm involved in bitemark casework that led to several wrongful convictions worldwide.[2-4] Specifically in bitemark cases, the uniqueness of human dentition consists of essential characteristics that are used for identification of perpetrators. These perpetrators are tracked by matching human dentition with patterned injuries. This procedure is not fully reliable or indisputable as long as the uniqueness of human dentition is still a matter of discussion. Currently, the legal integrity of convicted innocents is assured by special organizations such as The Innocence Project.[5] From a scientific view, the uniqueness of human dentition remains uncertain, which necessitates major efforts to support bitemark evidence.[1] There is ongoing improvement of imaging tools in engineering and graphic design. This indicates potential approaches for analysis of dental morphology and optimal approaches for investigations of the uniqueness of human dentition.[1] The present research sought to compare three existing software packages for 3D analysis of laser-scanned dental models.

The present research was designed as a cross-sectional experiment approved by the National Committee of Ethics in Research. The sample consisted of 20 human dental models randomly selected. The dental models were laser-scanned using the xCAD 3D® automated motion device. The obtained 3D models had a resolution of <20 microns. The Geomagic® Studio®, Cloud Compare®, and Maestro 3D Ortho Studio® software programs were tested for their metric and superimposition performances. In superimpositions, landmarking and cropping procedures were assessed. A blind test was included, simulating a real forensic case in which identical dentitions were merged for identification into a pool of randomly selected models. Intra- and inter-examiner calibrations were performed before the experimental steps. Statistical tests consisted of Dahlberg's error, applied to correlate the total variance with the error variance; Pearson's correlation coefficient and reliability coefficient, applied to assess the correlation of two sets of paired data; and Student's *t*-test, applied to investigate the level of discrepancy between two sets of paired data within a confidence interval of 95%. A qualitative analysis was performed exposing individual advantages and limitations of the three software programs based on an established quality standard.

Intra- and inter-examiner calibration reached >96.56% agreement for metric analyses and 95.27% agreement for superimposition analyses, without statistically significant differences between the examiners (p>0.05). The superimposition of dental models determined the error variance only in the landmarking procedure (p=0.01), indicating that the cropping procedure did not influence the final outcomes. The blind test provided 42 possible combinations of dental models. Identical models were properly distinguished from the remaining dataset with no mean discrimination and standard deviation <0.07mm. Cloud Compare® and Geomagic® Studio® achieved optimal performances considering the research purposes. Due to the software performance, free acquisition of Cloud Compare® was considered the most advantageous for applying the established quality standard. The present research indicated that existing software packages may usefully be applied to perform 3D comparisons of dental crown morphologies. Specifically, Cloud Compare® and Geomagic® Studio® software have good potential for superimposition analyses and evident advantages for metric analyses and comparisons.

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Uniqueness Human Dentition, 3D Scanning, 3D Superimposition

G56 Manipulation of Forensic Experts — Altering the Course of Criminal Justice in Hungary

Armin A. Farid, DDS, Podmaniczky Utca 33, III Fl, 8, Budapest 1067, HUNGARY*

After attending this presentation, attendees will better understand the importance of ensuring the authenticity of forensic reports in determining the fate of criminal investigations, particularly in cases involving bitemark analysis. Forensic dentists serving as experts are often pressured, by suspects and officials alike, to modify their findings to the advantage of the parties involved.

This presentation will impact the forensic science community by highlighting that their impartial and strictly objective standards are never to be compromised, under any circumstances, no matter how much pressure is exerted to bend the truth.

Despite its long history in educational and theoretical fields in Hungary, human bitemark analysis is increasingly integrated into police investigation work today. Since 2008, continuous media campaigns and educational programs put into place for the purpose of training Hungarian crime scene technicians have helped raise awareness of the importance of bitemark analysis among the police and the general public, resulting in the reporting of several bitemark cases within a short span of time. Almost all private and public television channels in Hungary covered reports and documentaries on bitemarks, bitemark analysis, implications in solving criminal cases, and educating the public of its value in proving a suspect's guilt or innocence.

In two separate cases, a forensic odontologist was approached and prompted to alter his findings in order to exonerate the suspects. One case involved a husband who claimed he was bitten by a drunk friend at a bachelor party, whereas his wife suspected the bitemark was acquired while engaging in a sexual affair. Another case involved a dog owner fighting to keep her dog from being put to sleep, claiming the dog had only scratched when in fact he had bitten his victim in a vicious attack. Both clients retracted their request for bitemark analysis, realizing that the forensic expert would not be manipulated and the reports would be objective and not falsely altered for their benefit.

In conclusion, even though massive strides have been made in the advancement of forensic science in Hungary, corruption on the part of a few who try to take advantage still poses a threat to justice. The importance of safeguarding accurate forensic evidence goes hand-in-hand with honesty and the dedication of experts who engage in the admirable work of public service in upholding the highest standards of ethics, truth, and justice.

Manipulation, Guilt, Innocence

PATHOLOGY/BIOLOGY

H1 From 3 Years to 3,000 Years: Forensic Taphonomy of Human Remains From the Irish Peatlands

Esther Jack, MBBCh, Office of the State Pathologist, C/O Fire Brigade Training Centre, Malahide Road, Marino Dublin 3, IRELAND; Niamh A. McCullagh, MSc, 3 Beechwood Drive, Boreenmana Road, Cork, IRELAND; and Linda M. Mulligan, MBBCH, Office of the State Pathologist, C/O Fire Brigade Training Centre, Malahide Road, Dublin Dublin 3, IRELAND*

The goals of this presentation are to: (1) present the forensic analysis of three cases of remains recovered from Irish peatlands from the perspective of both forensic pathology and forensic archaeology; and, (2) provide an overview of the forensic taphonomy of human remains in the context of waterlogged anaerobic acidic environments.

This presentation will impact the forensic science community by providing a knowledge basis for future investigations into the micro-environment of peatlands and their effect on postmortem decomposition. This presentation illustrates the value of a multidisciplinary approach to death investigation, particularly when dealing with concealed remains.

Introduction: Bog bodies are human or animal remains with preservation of non-bony tissue. Since the 17th century, Northern European peatlands have revealed the remains of more than 1,400 individuals dating from prehistory up to recent times. Preservation of these remains can be staggering. The reasons for this lie in the specific physical and biochemical composition of the bogs and of the bodies themselves.

This study examines the postmortem results of three cases of human remains exhumed from Irish peatlands ranging in date from 3 years to 3,000 years of age. This study is uniquely presented from the combined perspectives of both forensic pathology and forensic archaeology.

Methods and Materials: Several types of peatland environments occur throughout Northern Europe. The cases in this study were exhumed from raised bogs which generally occur 130m below sea level and would expect an annual rainfall between 800mm and 900mm. The remarkable preservative qualities of peatlands are attributed to a number of conditions including temperature, waterlogged anaerobic conditions, acidic pH, and antimicrobial factors (e.g., the polysaccharide sphagnan found in sphagnum moss).

This study presents three cases from departmental files in which human remains have been recovered from these unique environments. Included in this presentation is a discussion of the postmortem changes, their association with the environment, and pertinent external findings. Identifying features have been excluded to ensure anonymity.

Cases: Postmortem reports, images, and ancillary investigations from human remains exhumed after 3 years, 30+ years, and nearly 3,000 years were reviewed. The most recent case (Case 1) retained soft tissue, hair, and nails and external injuries and identifying marks were visible. There was also prominent adipocere formation and autolysis of organs. Case 2 from more than 30 years ago showed complete loss of soft tissues; however, adipocere formation was also prominent. Hair bearing skin was still attached to the skull. While the skeleton was incomplete, there was adequate material for positive identification. The most extraordinary preservation occurred in the remains exhumed from peatland after nearly 3,000 years (Case 3). Soft tissues and organs were retained with readily identifiable external marks and injuries; however, adipocere was not present in this case.

Discussion: Decomposition is a sophisticated multi-step process that takes place at macroscopic and cellular levels, eventually returning the constituents of a lifeless body to the ecosystem. Clothing and the body itself play significant roles in the degree of preservation. The outer integument and keratin structures are frequently the only identifiable surviving structures — these structures are remarkably well defined in Case 1. Adipocere formation is the result of the interaction of fat with a watery environment and was seen in the more recent cases (Case 1 and Case 2). Bony destruction is mainly a consequence of decalcification, a sequestering process which occurs more rapidly in the acidic conditions of peat bogs. Even in the advanced state of decay, the body surface may reveal evidence of trauma. Identification using various techniques was possible in all three cases.

Conclusion: This presentation provides a unique review of three cases of bog bodies recovered from Irish peatlands, buried for approximately 3, 36, and nearly 3,000 years, respectively. Each bog is unique in its micro-environment with variations in soil chemistry that lead to variations in preservation and survival of organic remains. Special emphasis is provided on their decomposition and preservation status, along with other anthropological discoveries relevant to their time. The preservative factors of peat environments are not completely understood. This presentation provides a knowledge basis for further studies involving the processes and factors influencing the decomposition of bodies buried in peatlands.

Bog Bodies, Forensic Taphonomy, Forensic Pathology

H2 Assessment of Infrared (IR) Thermography for the Estimation of Postmortem Interval in Rats

Jason W. Brooks, VMD, PhD, The Pennsylvania State University, Animal Diagnostic Laboratory, Wiley Lane, University Park, PA 16802; and Stephen Lynch, PhD, The Pennsylvania State University, Department of Mechanical and Nuclear Engineering, 331 Reber Bldg, University Park, PA 16802*

After attending this presentation, attendees will better understand the potential utility of an additional tool for use in the estimation of the postmortem interval. Attendees will be able to better design additional research studies based on IR thermography for the estimation of the postmortem interval in various species.

This presentation will impact the forensic science community by contributing to the body of literature a controlled study on the use of IR thermography for the measurement of temperature decay in animal carcasses. Although results of this study demonstrated that the rat body size was not significantly large enough to allow for differences in internal versus external cooling rates, additional research can now be based on this model using animals of larger body size.

Experimental measurements of body temperature decay versus time for euthanized rats were performed using an IR camera in order to understand the usefulness of external temperature data in the estimation of postmortem interval. Experiments were performed in a fume hood to provide controlled conditions by limiting IR radiation contamination and minimizing the effect of stray air currents. Thermocouples were used to capture ambient air temperature, rat internal body temperature, and rat external body temperature as a function of time. Four adult female Sprague Dawley rats (*Rattus norvegicus*) of approximately 280g body weight were euthanized by carbon dioxide asphyxiation, immediately instrumented with thermocouples, placed in the fume hood, and the IR camera and temperature data logger were started. Probe thermocouples were placed in the rectum through the anus, in the liver through a stab incision in lateral body wall, and in the brain through a hole drilled in the calvarium. A surface thermocouple was also attached to the abdominal skin. An external thermocouple was placed approximately 0.5 meters from the rat to register ambient air temperature in the hood. All thermocouple signals were captured by a data logger at a rate of one sample per minute. An IR camera set up vertically above the rat acquired whole-body images at one-minute intervals corresponding to the thermocouple samples. Test duration was approximately 14 hours, which was sufficient time for the rat body to cool to room temperature. Images were calibrated by comparing the temperature of the surface thermocouple in the IR image to the temperature measured by the data logger.

An initial analysis of the rat body temperature decay was performed by modeling the rat body as a convectively cooled cylinder with a circumference that was the average of four rat thoracic/abdominal circumferences and a length that was the average of the four rat bodies from the base of the neck to the base of the tail. This analysis neglected the contribution of the head to the cooling rate. The convective cooling in the fume hood-controlled conditions was estimated to be primarily natural (free) convection, in which the localized heating of the air by the body resulted in density differences that made the hot air rise and circulate. The method of analysis of the rat body temperature decay with time can be determined by examining the Biot (*Bi*) number, defined as the rate of convection over the rate of conduction in the body, given the characteristic length (L_c) of the body calculated as volume/surface area. Results demonstrated that the rat body L_c is small enough that the *Bi* number is approximately 0.1, signifying that the core body temperature does not differ significantly from the external body temperature. This implies that the use of IR thermography as an additional data point in the improvement of postmortem interval estimates for a rat are not likely useful, since it does not provide unique information beyond that provided by internal thermocouples; however, for a body with a larger L_c, the additional information provided by IR thermography could be helpful in providing additional temperature decay data. As an example, L_c for a human body is approximately an order of magnitude larger than that of the rat, thus Bi approximates 1.0 so the surface temperature would appreciably differ from the core temperature during cooldown.

In conclusion, these data suggest that IR thermography may provide useful temperature decay data in animals of body size that approaches that of an average adult human.

Postmortem Interval, Infrared Thermography, Temperature

H3 Blood-Derived Biomarkers for Estimation of Postmortem Interval (PMI)

Isabel Costa, MS, Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, Porto 4200-319, PORTUGAL; Teresa Magalhães, PhD, Faculty of Medicine, University of Porto, Porto, PORTUGAL; Paula Pinho, PhD, Faculty of Pharmacy, University of Porto, Rua José Viterbo Ferreira nº 228, Porto, PORTUGAL; Félix Carvalho, PhD, Faculty of Pharmacy, University of Porto, Rua José Viterbo Ferreira nº 228, Porto, PORTUGAL; Ricardo Silvestre, PhD, Life and Health Sciences Research, Un. of Minho, Braga, PORTUGAL; and Ricardo Jorge Dinis-Oliveira*, Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, Porto 4200-319, PORTUGAL*

The goal of this presentation is to increase the knowledge base of forensic pathologists investigating PMI estimation.

This presentation will impact the forensic science community by presenting the availability of two mathematical models that may have predictive value and become important complementary tools to traditional methods, thus achieving a more accurate PMI estimation.

A precise estimation of the *Postmortem* Interval (PMI) is one of the most important topics in forensic pathology however, the PMI estimation is based mainly on the visual observation of cadaverous phenomena (e.g., *algor*, *livor*, and *rigor mortis*) and on alternative methods such as thanatochemistry that remain relatively imprecise.[1-7] The goal of this *in vitro* study was to evaluate the kinetic alterations of several biochemical parameters (i.e., proteins, enzymes, substrates, electrolytes, and lipids) during putrefaction of human blood. For this purpose, a kinetic biochemical analysis of 46 parameters during a 264-hour period was performed. In an attempt to assure the maximum significance between each biochemical parameter and the putrefaction time, this study considered the posterior analysis of only those with Pearson correlations higher than 0.900 (i.e., in absolute value or modulus) and found to be statistically significant. Results showed a significant linear correlation between total and direct bilirubin, urea, uric acid, transferrin, Immunoglobulin M (IgM), Creatine Kinase (CK), Aspartate Transaminase (AST), calcium, and iron with the time of blood putrefaction. These parameters allowed development of two mathematical models that may have predictive value and may become important complementary tools of traditional methods to achieve a more accurate PMI estimation. For estimating the PMI in real samples, the average signals obtained from selected parameters with positive and negative slope () are used to calculate the value of *x*. The PMI should be calculated by the mean of the two equations. It is important to mention that the proposed model can match the most commonly found environmental conditions after death. Indeed, this study followed the *postmortem* decline of the body temperature model previously described by Siegel; however, this model is influenced by several internal (or intrinsic) and external (or extrinsic) *antemortem* and *postmortem* conditions, such as age, gender, xenobiotic administration, cause of death, body mass, duration of agonal state, environment temperature, humidity, rain, clothing, location of the body, and insect or animal activity.8 Therefore, the initial value as well as the slope of the curve will depend on many factors, which means that estimation of the time since death only reveals a certain period of time, but not a precise time point. Nevertheless, it was impossible to attain all potential variables and therefore this represents a preliminary study that must be further validated. It is expected that the current study may provide a new paradigm for estimation of PMI and become a complementary procedure for the methodologies already in use.

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Postmortem Interval, Blood Putrefaction Changes, Biochemical Parameters

H4 Decomposition of Mouse Carcasses Infected With Fluorescently Labeled Bacteria Provide Insight on Postmortem Microbial Translocation

Zachary M. Burcham, BS, Mississippi State University, 110 W Wood Street, Apt 12A, Starkville, MS 39759; Jennifer L. Pechal, PhD, Michigan State University, 243 Natural Science Bldg, East Lansing, MI 48824; Jeffrey L. Bose, PhD, University of Kansas Medical Center, 4003 Wahl Hall, W, Kansas City, KS 66160; M. Eric Benbow, PhD, Michigan State University, Depts of Entomology & Osteopathic Med Specialties, 288 Farm Lane, East Lansing, MI 48824; Carl J. Schmidt, MD, Wayne County MEO, 1300 Warren, Detroit, MI 48207; and Heather R. Jordan, PhD, Mississippi State University, PO Box GY, Mississippi State, MS 39762*

After attending this presentation, attendees will understand how host commensal microorganisms translocate and thrive immediately following the death and decomposition of the host. In addition, these microbial communities possess investigative potential during the discovery of remains in determining the postmortem interval.

This presentation will impact the forensic science community by providing original data that investigates how commensal bacterial populations translocate, colonize, and proliferate following death and successional decomposition of the associated host. Data obtained will significantly further investigations identifying specific microbial taxa or metabolic signatures for potential use in quantifiable, precise measurements of postmortem interval used in forensic science along with providing a visual fluorescent representation of bacterial translocation.

Microbially mediated mechanisms of human decomposition begin immediately after death and are a driving force for conversion of a once-living organism to a resource of energy and nutrients. Little is known about postmortem microbiology in cadavers, particularly the microbial structure of microflora residing within the human ecosystem, and their associations with decomposition stages. Recent work suggests that these bacterial communities are surprisingly dynamic during the postmortem interval.

This presentation describes how the microbiome of a living host changes and translocates within the body after death, linking the microbiome of a living being to the postmortem microbiome changes, which have demonstrated such promise as usable evidence in criminal investigations. The postmortem microbial community structure and function of *Staphylococcus aureus* (aerobic) and *Clostridium perfringens* (anaerobic) in the animal model *Mus musculus* (mice) were investigated to study how translocation of bacterial species can aid in the determination of postmortem intervals. The immunocompetent mice were inoculated nasally with fluorescently labeled *S. aureus*-Red Fluorescent Protein (RFP) and *C. perfringens*-Cyan Fluorescent Protein (CFP). A subset of mice was immediately surface sterilized with a 10% bleach solution following sacrifice and compared to non-surface sterilized mice in order to determine the influence of external microbiota. Both labeled bacteria were tracked using *in vivo* and *in vitro* imaging and analysis of gene expression to determine colonization routes and bacterial regulation response associated with different physiological events of host decomposition with time points starting at one hour and ending at 60 days. DNA was isolated from mice tissue samples that were preserved in DNA-RNA shield. The resulting DNA was purified for library preparation and whole genome shotgun sequencing.

These methods provide original data to uncover how commensal bacterial populations translocate, colonize, and proliferate following death and successional decomposition of the associated host. Data obtained significantly furthers investigations identifying specific microbial taxa or metabolic signatures for potential use in quantifiable, precise measurements of the time of death used in forensic science.

Microbial Translocation, Commensal, Postmortem Interval

H5 A Meta-Analysis of Carcass Decomposition on O'ahu, Hawaii

Alexis J.L. Peterson, Chaminade University of Honolulu, Forensic Sciences Unit, 3140 Waialae Avenue, Honolulu, HI 96816; Whitney A. Kodama, BA, Chaminade University of Honolulu, Forensic Sciences Unit, 3140 Waialae Avenue, Honolulu, HI 96816; and David O. Carter, PhD, Chaminade University of Honolulu, Forensic Sciences Unit, Honolulu, HI 96816*

After attending this presentation, attendees will understand how carcass decomposition is affected by seasons in a tropical climate.

This presentation will impact the forensic science community by demonstrating that although there may be temperature differences between seasons, this may not affect carcass decomposition.

When bodies are not discovered in the early postmortem period, they will decompose. Often decomposition leads to loss of physical evidence that increases the difficulty of identifying the deceased or establishing the cause of death; however, some postmortem changes introduce new forms of evidence. For example, fixed lividity can be used to establish body positioning. To best use postmortem changes as physical evidence, investigators must understand how they are influenced by the environment. This need has prompted several decomposition experiments to investigate the relationships between carcasses, environment, and decomposition; however, few studies have been conducted to investigate seasonal variation of carcass decomposition. The current study is a meta-analysis of environmental and decomposition data for summer and winter months on the island of O'ahu in an attempt to understand how postmortem changes are influenced by season.

Data from three previous decomposition experiments were analyzed to test the null hypothesis that carcass decomposition in the summer is not different than in the winter. Decomposition experiments were conducted during two summers (June 2013 and June 2014) and one winter (December 2014-January 2015). Each of the experiments was replicated three times with a total of nine swine (*Sus scrofa domesticus*) carcasses comprising the current dataset. To assess decomposition, mass loss and Total Body Score (TBS) were measured; mass loss was not measured in the Summer 2013 study. To further characterize decomposition, the pH, oxidation-reduction potential, and temperature of larval masses on all carcasses were also measured. Environmental temperature and relative humidity were measured at intervals of one hour during the course of decomposition. Accumulated Degree Days (ADD) were calculated using 0°C as base temperature. Descriptive and inferential statistics were generated using Prism 6 for Windows®, Version 6.05. Ambient temperature and relative humidity data were compared using a one-way Analysis Of Variance (ANOVA). Carcass mass loss, TBS, pH, oxidation-reduction potential, and larval mass temperature were compared using two-way ANOVA with Tukey's multiple comparisons test.

Ambient temperature in the summer experiments was significantly ($P < 0.001$) greater than in the winter experiment; however, no significant difference was observed in relative humidity. Although this difference in ambient temperature was observed, carcass decomposition was not significantly different between seasons. Similarly, a significant seasonal effect on larval mass temperature, pH, and redox was not observed. Carcasses lost approximately 80% of their mass during the experiment, which occurred by 275 ADD. Little mass was lost after this time. TBS also reached a maximum value (25), but this occurred by 150 ADD. The chemistry of the larval masses in all experiments was characterized as a warm, reducing, relatively neutral environment with temperature ranging from 30°C-40°C, pH values from 6.5-7.5, and oxidation-reduction potential from -100 millivolts to -300 millivolts.

These data show that carcass decomposition at this study site was not significantly different between summer and winter. The study's null hypothesis that carcass decomposition does not differ between summer and winter is accepted by this research. These results are surprising because the ambient temperatures between summer and winter were significantly different; temperature is well established as an important modulator of decomposition processes. The lack of significant difference in decomposition between seasons can be attributed to the tropical climate as it might not get sufficiently cold to slow carcass decomposition significantly. Another possible explanation is that the temperature of the larval masses, similar in all seasons, was able to compensate for the drop in ambient temperatures during the winter. A particularly interesting observation was that a maximum TBS was reached before a maximum mass loss. This has been interpreted to mean that some components of decomposition processes are not accounted for by TBS, an indirect measure of decomposition based on gross postmortem change. Although the current data are insightful, additional experimental studies will be conducted to further understand the seasonal dynamics of decomposition on O'ahu.

Total Body Score, Taphonomy, Season

H6 Analysis of Possible Impact Factors on the Regeneration Process of Hematomas in the Subcutaneous Fatty Tissue

Kathrin Ogris, MA, Medical University of Graz, Universitaetsplatz 4/2, Graz, Styria 8010, AUSTRIA; Thomas Widek, Ludwig Boltzmann Institut Clinical-Forensic Imagin, Universitaetsplatz 4/2, Graz, Styria 8010, AUSTRIA; Eva M. Hassler, Medical University of Graz, Auenbruggerplatz, Graz 8036, AUSTRIA; Patrick P. Torreiter, Medical University of Graz, Auenbruggerplatz, Graz 8036, AUSTRIA; Andreas Petrovic, MSc, Graz University of Technology, Kronusgasse 5, Graz 8010, AUSTRIA; and Eva Scheurer, MD, Institut für Rechtsmedizin, Pestalozzistr.22, Basel 4056, SWITZERLAND*

After attending this presentation, attendees will recognize that dating of hematomas in the subcutaneous fatty tissue is particularly important for the reconstruction of criminal acts, such as child abuse cases, where accurate timing of injuries can define or at least set limits on a period of time during which a crime took place. Hence, accurate timing of injuries can lead to an inclusion or exclusion of potential suspects.

This presentation will impact the forensic science community by underlining the importance of radiologic methods in forensic medicine.

In clinical forensic medicine, it is often important to determine the time of origin of soft tissue injuries. As subcutaneous hematomas are usually not relevant for clinicians, only limited knowledge exists regarding the detection and dating of traumatic lesions in the subcutaneous fatty tissue using Magnetic Resonance Imaging (MRI).

However, visual assessment of external hematoma color, the currently used method for estimating hematoma age, is unreliable due to its great inter-observer variability and is affected by individually varying color perception.[1,2] Novel attempts at hematoma age estimation are striving to create a reliable and objective model using MRI. First studies showed that the contrast of hematomas in MRI can be used to obtain objective information on hematoma characteristics.[3] Based on these initial results, that the regeneration process of hematomas may also depend on various influencing factors such as hematoma shape or the structure of the subcutaneous fatty tissue, the goal of this study was to explore the impact of these and other potential factors on contrast behavior of subcutaneous hematomas over time in order to date traumatic lesions for the forensic reconstruction of events.

In 30 healthy volunteers (18 male, 12 female, age: 26.3±3.8 years) without coagulation disorders or medication influencing blood clotting, 4mL of autologous blood were injected into the subcutaneous fatty tissue of the thigh. MRI was performed directly after the injection and after 3, 24, 72, 168, and 336 hours on a 3T MR scanner using a multifunctional coil. The MR sequence protocol consisted of a Proton Density-weighted Turbo Spin Echo sequence with Spectral Adiabatic Inversion Recovery (PDwTSE SPAIR) fat saturation and a PDwTSE with water saturation (watersat) in oblique and axial orientation. Data were analyzed by measuring signal intensities in nine Regions of Interest (ROIs) (0.4cm^2), three each in the hematoma, fat, and muscle tissue. After pooling the measurements of each tissue, the contrast coefficient according to Michelson was calculated.[4] Additionally, visual evaluation of hematomas and lobular structure of the subcutaneous fatty tissue were performed. After identifying potential influencing factors, such as gender, age, Body Mass Index (BMI), fat lobuli structure, hematoma shape, and thickness of the subcutaneous fatty tissue, statistical analysis using non-parametric tests were performed. In these tests, the Michelson coefficients, grouped by the different influencing factors, were investigated at different points in time.

Two types of fat lobuli (spherical/fusiform) and two different shapes of hematomas (compact/diffuse) were identified. Diffuse hematomas were more frequently seen in women than in males and were associated with the thickness of the subcutaneous fatty tissue layer of more than 1cm in the thigh. In females, diffuse hematomas were associated with spherical fat lobuli and compact hematomas with fusiform lobuli, while in men only fusiform lobular structure could be found, showing both types of hematoma shapes. Statistical significant differences of the contrast behavior of the hematomas versus fat over time were only found when dividing the participants into two groups regarding their BMI (normal weight, overweight).

Several factors were assumed to be influencing on the hematoma regeneration process; however, in artificial hematomas investigated over two weeks, only the BMI was identified as a significant factor for differences in the contrast behavior over time.

Nevertheless, one has to take into consideration that actual hematomas might differ concerning their regeneration process. Therefore, in a subsequent study with actual hematomas, the potential influencing factors need to be re-evaluated.

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Hematoma, MRI, Age Estimation

H7 A New Approach to Collecting, Fixing, and Preparing Samples for Sperm Cells in Cases of Alleged Rape

Helga Haahr-Lillevang, MD, Institute of Forensic Medicine, Brendstrupgårdsvej 100, Aarhus N 8200, DENMARK; Maria Pihlmann, MD, Institute of Forensic Medicine, Brendstrupgaardsvej 100, Aarhus N 8200, DENMARK; Anette M. D. Funder, Institute of Forensic Medicine, Brendstrupgårdsvej 100, Aarhus N 8200, DENMARK; Marianne S. Martiny, MA, Institute of Pathology, Aarhus University Hospital, Aarhus C, DENMARK; Tine H. Meyer, MA, Institute of Pathology, Aarhus University Hospital, Aarhus C, DENMARK; and Iana Lesnikova, MD, PhD, Havkaertoften 14, Tilst 8381, DENMARK*

After attending this presentation, attendees will be introduced to a new approach of collection, Alcohol-Based Fixation (ABF), and preparation of samples suitable for both Hematoxylin-Eosin (HE) and immunohistochemical detection of sperm cells.

This presentation will impact the forensic science community by introducing the ABF technique, allowing a faster and easier visualization of sperm cells in cases of alleged rape.

In cases of alleged rape, the early detection of sperm cells has great importance, since it strongly indicates that a sexual act has taken place. Like most forensic laboratories, the laboratory of the Institute of Forensic Medicine, University of Aarhus, Denmark, in all cases of alleged rape and suspected sexual assault over the years has routinely performed the light microscopy of HE-stained cytological smears. Samples have been collected using dry cotton swabs, spread over glass slides, air dried, and then proceeded to HE staining and light microscopy. This method has clear benefits: it is quick to perform, very inexpensive, and does not need any advanced laboratory facilities. The major limitation is that there is only a single glass slide available and there is no possibility for additional stains. Another known limitation of this method is a relatively large amount of time spent in evaluation of slides due to difficulty in identification of sperm cells covered by layers of overlaying cells, crush artifacts, and difficulty in distinguishing sperm cells from cellular debris, lymphocytes, and clusters of bacteria and fungi.

Test sample series and one control sample were obtained from four anonymous healthy female volunteers of childbearing age. Samples were taken after intercourse and for the following three days. Vaginal fluid samples were obtained by the volunteer using a vat tip swab. Swabs were briefly soaked in a tube containing 1ml of fixing solution and were then disposed. The corresponding conventional, air-dried smear samples were also obtained. Two alcohol-based fixing solutions (58% ethanol solution and commercially available methanol-based buffered CytoLyt® solution) were tested. Sample slides were prepared using the Cytospin technique, which is a well-established cytology method that is specifically designed to concentrate cells such as those that are found in small numbers. HE and Immunohistochemical (IHC) stains using a SPERM HY-LITER™ kit were performed and evaluated at light microscopy.

The sperm cells were better preserved in CytoLyt® fixed samples, but the difference was negligible. In all ABF and conventional smears taken less than 24 hours after intercourse, sperm cells with tails were found. In two ABF samples (taken within 48-72 hours and within 72-96 hours after intercourse, respectively), sperm cells were found only in conventional slides but not in ABF samples. In one sample, taken within 24-48 hours after intercourse, a few sperm cells without tails were in the ABF sample but not in conventional slide. The IHC staining was unproblematic in all ABF samples. The evaluation time was significantly lower in ABF samples.

This result showed the ABF technique is suitable with both HE and IHC staining procedures. The level of detection of sperm cells in ABF-based slides seemed to be similar to conventional smears, but the ABF technique has provided faster, easier visualization of sperm cells.

Forensics, Sperm Cells, Cytology

H8 A Preliminary Study of Shifting Bacterial Communities of the Face During Human Cadaver Decomposition in Southeast Texas

Lauren R. Smith, BS, Sam Houston State University, 2830 Lake Road, #1203, Huntsville, TX 77340; Joseph F. Petrosino, PhD, Baylor College of Medicine, Dept of Molec Virology & Microbiology, Houston, TX 77030; Sibyl R. Bucheli, PhD, Sam Houston State University, Dept of Biological Sciences, Box 2116, Huntsville, TX 77340; and Aaron M. Lynne, PhD, Box 2116, LDB #300, 1900 Avenue I. Huntsville, TX 77341*

After attending this presentation, attendees will better understand fine-scale variations of population structures of the microbiome related to human decomposition.

This presentation will impact the forensic science community by providing standardization of sampling methods using in forensic research studies for the future application of estimating the postmortem interval. The presented data can help validate similar studies as well as fine-tune protocols currently used in related research.

Human decomposition is a process marked by events categorized into five stages. These stages occur as a fluid procession rather than through precise demarcation of events and may lead to cadavers experiencing multiple stages of decomposition at once. Previous studies have investigated biodiversity of necrophagous bacteria and insects at predefined stages of decomposition with a focus on taphonomy. One area yet to be explored is fine-scale temporal and spatial influence on these findings. The pilot studies and early data have shown that there are shifts in the microbiome present on the skin of human cadavers, which also follow the shifts in stages of decomposition.

As part of a separate ongoing study, two human cadavers were placed outdoors at the Southeast Texas Applied Forensic Science (STAFS) facility in Huntsville, TX, on July 31, 2013. The face of each cadaver was divided into a grid to be sampled to ascertain the spatial and temporal influence that fine-scale sampling can have on bacterial community structures. These cadavers were sampled at 18 locations on the face every six hours over the course of four days. The samples were subject to 16S rRNA gene sequencing using the Illumina® platform, then analyzed using the Quantitative Insights Into Microbial Ecology (QIIME) software. Results from the fine-scale study follow different initial trends but mirror trends that have been observed during the larger scale analyses. Cadaver STAFS 2012.035 follows the trend of human-associated bacteria (*Staphylococcus* and *Pseudomonas*) shifting to insect-associated bacteria (*Ignatzschineria* and *Wohlfhartiimonas*) that then shifts to soil-associated bacteria (*Sporosarcinia*). Cadaver STAFS 2013.026 follows a similar trend with a surprising interval of *Clostridium* dominating the sample set between the initial samples and the time when insect-associated bacteria is dominant in the samples.

While these two cadavers do not follow the same trend of shifting bacterial communities, the data helps to bring insight into the overall goal of understanding bacterial succession during human decomposition. Over the course of a single day, these data show that time of day likely plays a factor in the bacterial community composition in a sample. Between sample locations, there is also indication of differences in bacterial communities. The significance of these differences will determine future sampling techniques as well as help standardize the way in which the microbiome of human decomposition is studied and used as a predictive model for estimating the time since death. The overall goal of studying the microbiome of human decomposition is to provide another tool for estimating the postmortem interval for forensic applications and investigations.

Human Decomposition, Microbiome, Postmortem Interval

H9 Bacteria Triggering a Preference in Flesh Flies (Diptera: *Sarcophagidae*) Associated With Human Cadavers

Keli L. King, Sam Houston State University, 2200 Crosstimbers, #3, Huntsville, TX 77320; Aaron M. Lynne, PhD, Box 2116, LDB #300, 1900 Avenue I, Huntsville, TX 77341; Sibyl R. Bucheli, PhD, Sam Houston State University, Dept of Biological Sciences, Box 2116, Huntsville, TX 77340; and Joseph F. Petrosino, PhD, Baylor College of Medicine, Dept of Molec Virology & Microbiology, Houston, TX 77030*

After attending this presentation, attendees will have a better understanding of insect interactions with human cadavers.

This presentation will impact the forensic science community by helping attendees understand the interaction between bacteria found on the decomposing cadavers and flesh flies which may provide a deeper understanding of how decomposition progresses and, if chemical compounds are responsible for fly attraction, they may also help form a more precise estimation of the Postmortem Interval (PMI).

Bacteria, credited as having a major role in human decomposition, are credited with driving the tempo and mode of the process. Flesh flies of the family *Sarcophagidae* ("flesh-eating") are among the first scavengers to arrive to a cadaver and can greatly impact soft tissue removal, along with other flies, and may be responsible for delivering important bacteria to the ecosystem. Flesh flies are attracted or repulsed by various stages of decomposition, aiding in the establishment of a cadaver-specific microbiome. Understanding the interaction between bacteria found on the decomposing cadavers and flesh flies may provide a deeper understanding of how decomposition progresses, and if chemical compounds are responsible for fly attraction, they may help to form a more precise estimation of the PMI.

In an effort to understand fly behavior, this research tested the flies' preference for bacteria specific to these stages of decomposition. Using classic choice experiments, swabs of bacteria from two decomposing cadavers placed outdoors to decompose under natural conditions at the Southeast Texas Applied Forensic Science (STAFS) facility, a willed body donation facility at Sam Houston State University in Huntsville, TX, were presented to commercially purchased flies from April and July of 2015. Sterile cotton-tipped applicators were used to swab cadavers at different stages of decomposition (initial decay, putrefaction, black putrefaction, butyric fermentation, and dry decay) and at different areas of the body (mouth, groin area, and torso). The swabs were placed into respective sterile cryotubes and stored at -80°C. A control experiment was performed using the same stage of decomposition, the same body sites, and the same storage method of the cadaver on both ends of the y-tube. Swabs were placed at the top of the y-tube thereby giving flies a choice as to which stage they prefer. A chi-squared distribution was used to determine if preference was due to random chance alone. Taxa summaries showing bacterial community structures were created from sequenced data at each sample site for each stage of decomposition. These were used with the data from fly preference trials to correlate preference to stage-associated bacteria.

Preliminary results suggest that flies have a preference for the consortium of soil, human, and fly bacteria associated with initial stages of decomposition. This research has significance in forensic sciences. If the initial consortium of bacteria is altered (e.g., freezing), it could interfere with fly oviposition behavior and lead to a skewed estimation of PMI. Ultimately, bacterial data such as these can be refined to develop a model of microbial succession that can then be used to estimate the postmortem interval or the time since death.

Flesh Flies, Human Decomposition, Insect Behavior

H10 The Six Little Pigs: Estimation of Long-Term Postmortem Interval (PMI) Based on Bacterial Community Succession in Porcine Remains

Michael S. Woolf, BS, Virginia Commonwealth University, 6732 Hopton Court, Richmond, VA 23226; Vanessa Sufrin, MS, 11507 Harvestdale Drive, Fredericksburg, VA 22407; Baneshwar Singh, PhD, Virginia Commonwealth University, Dept of Forensic Science, 1015 Floyd Avenue, Rm 2015, Richmond, VA 23284; and Tal Simmons, PhD, Virginia Commonwealth University, Dept of Forensic Science, 1015 Floyd Avenue, Richmond, VA 23284*

After attending this presentation, attendees will better understand porcine decomposition, the roles of bacteria and insects during the process, and how bacterial community succession associated with porcine remains can be useful for human PMI estimation.

This presentation will impact the forensic science community by providing further insight into the potential usefulness of changes in relative bacterial abundance and, subsequently, more accurate long-term PMI estimation.

Bacteria, like insects, drive the vertebrate decomposition process.[1,2] Previous studies have demonstrated changes in human and porcine bacterial community structure over time.[1,3,4] In this study, six porcine cadavers were placed in an open field at the Virginia Public Safety Training Center (VPSTC) in Hanover, VA. Primary arthropod colonizers (e.g., blow flies, flesh flies, beetles, etc.) had free access to all six carcasses. Minimum 10m distance was maintained between any two carcasses. Sterile cotton swabs were used for microbial sample collections from skin regions of each cadaver. Swabs were collected daily through day 7 (T7), every other day through day 15 (T15), and once weekly through day 61 (T61). DNA was extracted using a phenol:chloroform:isoamyl alcohol organic extraction method.[5] Two variable regions (V3-V4) of the 16S ribosomal RNA (rRNA) gene were amplified using barcoded primer pair 341F and 806R for MiSeq® sequencing.[6]

Preliminary assessment of primary insect colonization showed that adult flies from family Calliphoridae (*Phormia regina*, *Lucillia spp.*, *Cochliomyia macellaria*) and beetles from families Silphidae (*Necrophila americana*, *Necrodes surinamensis*, *Oiceoptoma novaboracense*), and Staphylinidae (*Creophilus maxillosus*) were observed in large numbers during early decomposition stages (fresh and bloat). Egg masses, fly larvae, and a single rove beetle larva were observed and collected within 24 hours of pig placement. In addition to those aforementioned species, beetles from family Cleridae (*Necrobia rufipes*) were noted during later decomposition stages (active decay, advanced decay, and dry). Overall, *Pharma regina* and *Creophilus maxillosus* were the most prevalent fly and beetle species throughout this study. Maggot masses began migrating on or about days 3-4 with peak migration on days 7-8. Vultures were also observed at the test site on days 7 and 8.

In conclusion, this study will expand knowledge regarding bacterial community succession in porcine remains and will further elucidate the usefulness of bacterial succession associated with porcine remains in estimation of long-term PMI. This study will also help determine how bacteria associated with porcine remains change in different geographical regions, identify indicator bacterial species for each stage of decomposition, and clarify which species are common in all geographical locations.

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Bacteria, Forensics, Postmortem Interval

H11 Investigation of the Utility of Five Messenger RNA (mRNA) Markers (SEM1, KLK3, PRM1, PRM2, and TGM4) in the Identification of Semen

Ayse Serin, PhD, University of Cukurova, Faculty of Medicine, Dept of Forensic Medicine, Adana, TURKEY; Vugar K. Huseynov, PhD, 33, 20 January Street, Apt 58, Az1102, Baku, AZERBAIJAN; Husniye Canan, PhD, University of Cukurova, Faculty of Medicine, Dept of Forensic Medicine, Adana, AL, TURKEY; Ayca Ulubay, University of Cukurova, Faculty of Medicine, Dept of Forensic Medicine, Adana 01330, TURKEY; and Mete K. Gulmen, PhD, MD, Cukurova University, School of Medicine, Dept of Forensic Medicine, Adana 01330, TURKEY*

After attending this presentation, attendees will better understand the effect of mRNA profiling in the identification of semen.

This presentation will impact the forensic science community by illustrating that five semen mRNA markers could be used as an alternative to the traditional method in the identification of semen.

mRNA profiling is a promising method studied for nearly ten years in identifying biological fluids.[1-5] In this study, the utility of five mRNA markers (Protamine 1 (PRM1), Protamine 2 (PRM2), Semenogelin 1 (SEM), Kallikrein 3 (KLK3), and Transglutaminase 4 (TGM4)) in identification of semen was investigated. 18SRNA, Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), Transcription Elongation Factor (TEF), and Ubiquitin-Conjugating Enzyme (UCE) mRNA markers were used as endogenous control. RNAs were analyzed by reverse transcriptase and multiplex Polymerase Chain Reaction (PCR) method following combined isolation of DNA/RNA/micro RNA (miRNA) in order to investigate the rate of presence in semen, specificity and sensitivity of the selected markers, and if they were appropriate in defining stains kept under different environmental conditions. The products were run in capillary electrophoresis.

The five mRNA markers selected were identified in all of the 25 fresh semen samples and the rate of presence in semen was 100%. All markers could be detected in 0.1µl semen by multiplex PCR method. The most sensitive one among these markers was found to be SEM1 (the sensitivity was defined to be 0.0025µl). None of the five semen markers could be identified in samples of blood, menstrual blood, saliva, and vaginal fluid. It was found that the stabilities of the selected semen mRNA markers were proportional with their sensitivities. It was observed that the stability of TGM4, KLK3, and PRM2 in stains was lower compared to SEM1 and PRM1.

Fifteen semen stains which were kept for five years at 4°C, room temperature (22-25°C, humidity ~60%), and 37°C in paper envelopes were used for the goal of testing time-dependent stability. It was found that the RNA integrity of the samples kept for the same period at a lower temperature, but in a damp environment (room temperature), disrupted much faster compared to the ones which were kept in dry, hot weather (37°C in incubator).

A full DNA profile could be achieved in 0.04µl of semen in which DNA/RNA were isolated in combination and a partial DNA profile could be achieved in 0.01µl of semen by using a commercial 15 Short Tandem Repeat (STR) PCR amplification kit.

The results showed that both multiplex DNA profile and semen-specific multiplex RNA profile could be demonstrated in a single sample by isolation of DNA/RNA in combination in a very small amount of semen and the selected five semen markers could be used as an alternative to the traditional method in identification of semen because of their sensitivity, specificity, and stability in stains. Although the sensitivities of SEM1 and PRM1 were higher, it was thought that all five semen markers selected could be used in forensic semen identification by using multiplex PCR protocols.

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Semen Identification, mRNA Profiling, Body Fluid Identification

H12 Blow Flies and Nicotine: An Entomotoxicology Study

Paola A. Magni, PhD, University of Western Australia, Crawley, Western Australia 6009, AUSTRALIA; Marco Pazzi, PhD, University of Turin, Dept of Chemistry, Via P. Giuria n.5, Torino, ITALY; Eugenio Alladio, MS, Centro Regionale Antidoping "A. Bertinaria," Regione Gonzole 10/1, Orbassano (Turin) 10043, ITALY; Marco Vincenti, MS, Centro Regionale Antidoping, Regione Gonzole 10/1, Orbassano, Torino 10043, ITALY; Marco Brandimarte, MSc, University of Turin, Dept of Chemistry, Via P. Giuria n. 5, Torino, ITALY; and Ian Dadour, PhD, Boston University, Program in Forensic Anthropology, Dept of Anatomy & Neurobiology, Boston, MA 02118*

After attending this presentation, attendees will understand the potential capabilities of entomotoxicology and how the presence of nicotine, a common and powerful drug, can affect the survival and the developmental rate of blow flies and, as a consequence, have an effect on the Postmortem Interval (PMI) estimation.

This presentation will impact the forensic science community by providing data that will be potentially useful in adding a new component to estimating the minimum time since death of remains exposed to drugs.

Entomotoxicology is a branch of forensic entomology which studies the potential uses of insects for detecting drugs, other toxic substances, and gunshot residues in decomposing tissues. In fact, insects developing on a cadaver present an alternative and appropriate substrate for analysis of toxicological substances, especially in the absence of tissues or biological fluids. The main focus of forensic entomology is determining the time elapsed since death of a deceased human or animal using arthropods, but in recent studies it has been demonstrated that such time frames may be severely compromised by drugs and toxins. Therefore, entomotoxicology also investigates the effects of these substances in cadavers, on arthropod development, and morphology. While the detection of drugs, metals, pesticides, and alcohol has been reported in entomotoxicological studies, there is no research concerning the detection, analytical quantification, and the effect of nicotine on the necrophagous entomofauna.

Nicotine is a volatile and water-soluble alkaloid present in the tobacco plant (*Nicotiana* species, Solanales: Solanaceae). Nicotine has acute toxicity and it is considered one of the most deadly poisons known to man: rapid administration of large doses of nicotine may cause death within a few minutes. Nicotine, regardless of the mode of administration, can be readily absorbed across the epithelium of the lung, the nose, and through the skin and mucosa. Therefore, there is a potential for poisoning from ingestion, injection, inhalation, and skin and rectum absorption of nicotine from nicotine-containing products.

Nicotine can be found in tobacco products (e.g., cigarettes, cigars, pipes, and refill solutions for e-cigarettes), in products for Nicotine Replacement Therapy (NRT), toothpastes, and insecticides. In humans, the median lethal dose (LD_{50}) of nicotine is 0.5mg/kg-1.0mg/kg, which means that the fatal dose of nicotine can be estimated in 30mg-60mg of nicotine in adults and 10mg in children. The nicotine content in an average cigarette is 10mg-20mg and the nicotine concentration in e-cigarette refills can reach approximately 22mg/mL; however, despite most of the everyday tobacco products containing a considerable amount of nicotine, only a small percentage can be absorbed by the body.

The literature reports a number of accidental/sudden, suicidal, and homicidal cases whereby nicotine (alone or mixed with other drugs) was used. To note, recipes are readily available on the internet on how to extract pure nicotine from smoking tobacco for suicidal purpose.

In the current research, the presence and the effects on larvae of the necrophagous blow fly *Calliphora vomitoria* L. (Diptera: Calliphoridae) were examined when reared on substrates (beef liver) spiked with three different concentrations of nicotine that could cause death in a human (2ng/mg, 4ng/mg, 6ng/m).

A method was developed and validated for Gas Chromatography/Mass Spectrometry (GC/MS) to determine nicotine in larvae reared on spiked liver. Statistics were calculated to determine the linearity of method, coefficient of determination (R2>0.99), the evaluated detection limit (LOD=0.25ng/mg), the quantification limit (LOQ=0.86ppb), extraction recovery percentage, precision, selectivity, and carry over. All parameters were satisfied.

The results demonstrated the following: (1) GC/MS can detect both nicotine and its metabolite cotinine in *C. vomitoria* immatures; (2) the presence of the three scheduled nicotine concentrations in the food substrate did not modify the developmental time of *C. vomitoria*; (3) during the pupation period, larvae reared on substrates containing nicotine died dependent on the concentration of nicotine present; and, (4) the resultant lengths of larvae and pupae exposed to both concentrations of nicotine were significantly shorter than the control.

Results of this research will improve the general knowledge in the field of forensic entomology/entomotoxicology as well as provide another tool that may be useful in an investigation; however, this research underlines the need for further studies concerning nicotine and blowflies, such as: (1) the effects of lower and higher nicotine doses on blowfly development; (2) how nicotine mixed together with other drugs affects blow flies; and, (3) nicotine metabolite pathways and their effects on blow flies.

Entomotoxicology, Nicotine, Calliphora Vomitoria

H13 Internal Validation of the Promega® PowerPlex® Y23 Amplification Kit for Use in Forensic Casework

Jordan L. Clarke, BS, 118 6th Avenue, Apt 1, Huntington, WV 25701; Jody West, NC State Crime Lab, 121 E Tryon Road, Raleigh, NC 27603; Kristin Meyer, MFS, NC State Crime Laboratory/NCDOJ, 121 E Tryon Road, Raleigh, NC 27603; and Pamela J. Staton, PhD, Marshall University Forensic Science MSFS & Center, 1401 Forensic Science Drive, Huntington, WV 25701*

After attending this presentation, attendees will understand Y-chromosomal Short Tandem Repeat (Y-STRs), the Promega® PowerPlex® Y23 PCR amplification kit, and the benefits the kit can provide if implemented in conjunction with traditional STR testing in forensic casework.

This presentation will impact the forensic science community by demonstrating the improved capabilities of Promega's® newest Y-STR system which includes greater efficiency and discrimination. The system can be used for both casework and direct amplification applications with a shortened amplification time at an hour and a half, as well as demonstrate a large degree of sensitivity, even in the presence of excessive female DNA.

Y-STR systems can be an effective tool in a variety of ways within the forensic DNA community. Most commonly, Y-STRs have demonstrated their usefulness in sexual assault evidence where the female DNA contribution overwhelms the male DNA contribution or when a differential extraction cannot effectively separate the male and female components. Generation of male profiles with Y-STRs can also assist in the identification of missing persons and human remains within the Combined DNA Index System (CODIS) in conjunction with traditional autosomal STR testing. Additionally, Y-STR testing can help in distinguishing paternally unrelated male contributors in complex autosomal DNA mixtures as well as potentially excluding male contributors in samples containing minor male components.

The PowerPlex® Y23 System is the most recent Y-STR system developed by Promega® to replace the previous Y-STR system, PowerPlex® Y. The PowerPlex® Y23 amplification kit contains 11 more loci than the previous Promega® Y system and includes two rapidly mutating loci which allows for potentially greater discrimination between paternally related males. The system can be used for both casework and direct amplification applications with a shortened amplification time of an hour and a half, providing a more efficient analysis process. The Y23 kit demonstrates a large degree of sensitivity, even in the presence of excessive female DNA.

The North Carolina State Crime Laboratory (NCSCL) Forensic Biology section does not currently use a Y-STR kit in casework processing. An internal validation was performed on the PowerPlex® Y23 PCR Amplification kit in accordance with the Scientific Working Group for DNA Analysis Methods (SWGDAM) validation guidelines and the Federal Bureau of Investigation (FBI) Quality Assurance Standards for Forensic DNA Testing Laboratories September 2011 revision. Automated extractions were performed through the project using the Qiagen® EZ1 Advanced® Robot. Quantitation was performed on the Applied Biosystems® 7500 Real-Time Polymerase Chain Reaction (PCR) instrument using the Quantifiler® Duo quantification kit and the GeneAmp® 9700 thermal cycler was utilized for PCR amplification. Capillary electrophoresis was performed on the Applied Biosystems® 3500xL Genetic Analyzer and all data were analyzed using GeneMapper® ID-X v 1.4.

Internal validation studies included the following: precision; sensitivity; concordance; reproducibility; contamination; stochastic; mixtures (to include male/male and male/female scenarios); stochastic evaluation of the DYS385 locus; minimum threshold assessment; and, non-probative/mock sample studies. Sensitivity results demonstrated that the Y23 system could consistently generate full profiles at concentrations of 0.03125ng and full male profiles were also observed in several samples at concentrations as low as 0.0156ng. Male/female mixture study results indicated that full male profiles could consistently be obtained at ratios as extreme as 1:16,000, illustrating the specificity of the Y23 kit for male DNA amplification. Additionally, a study was performed to explore the viability of the Y23 PCR product over a period of several weeks. These studies identified the most efficient and appropriate operating procedures for the PowerPlex® Y23 amplification kit that meets the laboratory's needs and requirements.

This internal validation demonstrates the potential benefit of implementing the PowerPlex® Y23 kit in other forensic casework laboratories and will assist the NCSCL's Forensic Biology section in evaluating the addition of Y-STR analysis in the analysis of sexual assault evidence. The NCSCL Forensic Biology section will perform future studies to include stutter assessment, half-reactions, and alternate injection times. Other studies looking at the correlation between high mutation rate loci and related male samples may also be performed. The implementation of the PowerPlex® Y23 system will expand the testing capabilities of the forensic biology section.

DNA, Y-STRs, Y23

H14 Study on Forensically Important Insects Collected From Medicolegal Autopsies in South Korea

Sang Eon Shin, Department of Legal Medicine, Korea University College of Medicine, 73, Inchon-ro, Seongbuk-gu, Seoul, Seongbuk-gu 02841, SOUTH KOREA; Im Joo Rhyu, PhD*, Department of Anatomy, Korea University College of Medicine, Seoul, SOUTH KOREA; Seong Ho Yoo, PhD*, Department of Forensic Medicine, Seoul National University College of Medicine, Seoul, SOUTH KOREA; Hyung Seok Kim, PhD, Department of Forensic Medicine, Chonnam National University Medical School, Gwangju, SOUTH KOREA; and Seong Hwan Park, PhD*, Department of Legal Medicine, Korea University College of Medicine, Seoul, SOUTH KOREA*

After attending this presentation, attendees will better understand the significance of forensically important insects related with human cadavers and the experimental methodology of other countries.

This presentation will impact the forensic science community by reporting on forensically important insects related with human cadavers in the East Asian temperate zone for the first time.

Forensic entomology is a branch of forensic science which applies insect evidence to legal problems mainly for the estimation of minimum Postmortem Interval (PMI-min). Knowledge of the necrophilous insect fauna of each geographic region is very important for the acceptability and credibility of insect evidence for forensic application. In South Korea, most research carried out during the past 20 years was confined to insect fauna attracted to animal carrion or DNA-based identification of necrophilous fly species; however, forensically important information about insects attracted to human cadavers in South Korea as well as the East Asian temperate climate zone is still unsatisfactory.

In the years 2010, 2011, 2013, and 2014, 1,602 insects were collected from 48 medicolegal autopsies in northeastern Seoul, South Korea, and its suburb. Insect infestation of human cadavers was most common in the summer and was not observed at all in January and February. After the families of maggots were identified by the shape of the posterior spiracles, DNA barcoding using nucleotide sequences of mitochondrial Cytochrome c Oxidase subunit I gene (COI) was performed for species identification. The adult individuals were identified to the species level by external morphological characters. As a result, 4 orders, 15 families, 23 genera, and 39 species were confirmed. The four orders identified were Diptera (6 families, 17 species), Coleoptera (6 families, 19 species), Hymenoptera (2 families, 2 species), and Dictyoptera (1 family, 1 species).

The dominant species in this study, *Lucilia sericata* (Order: Diptera; Family: Calliphoridae), showed a strong preference for indoor environments. Sarcophagidae species only occurred during the summer. The occurrence of *Piophila casei* (Family: Piophilidae), a potential indicator of an advanced stage of decay, was the first official identification of this species in South Korea. Another Piophilidae species, *Parapiophila vulgaris*, coexisted with *P. casei* in one case. Coleoptera species appeared entirely in the summer except for an individual *Dermestes haemorrhoidalis* (Family: Dermestidae) found in a greenhouse in December. The dominant beetle species in this study was *Dermestes maculatus* (Order: Coleoptera; Family: Dermestidae). Silphidae species were observed four times exclusively in the forest cases. Only 13 individuals were collected for families Histeridae, Staphylinidae, and Cleridae known to feed on maggots.

Because the sample size of this study was limited, more extensive sampling is required to characterize the forensically important beetle fauna in South Korea. Despite its limited scale, this study provides a snapshot of the general entomofauna attracted to human cadavers in this region. A more wide-scale survey for the entire Seoul metropolitan area and its suburb is ongoing from 2015.

Forensic Entomology, Autopsy, Entomofauna

H15 Analysis of an Additional Nine Short Tandem Repeat (STR) Loci to 15 STR Loci and the Detection of Allele Frequencies in a Cukurova Population of Turkey

Ayca Ulubay, University of Cukurova, Faculty of Medicine, Dept of Forensic Medicine, Adana 01330, TURKEY; Husniye Canan, PhD, University of Cukurova, Faculty of Medicine, Dept of Forensic Medicine, Adana, AL, TURKEY; Ayse Serin, PhD*, University of Cukurova, Faculty of Medicine, Dept of Forensic Medicine, Adana, TURKEY; Necmi Cekin, MD, Cukurova University School of Medicine, Dept of Forensic Medicine, Balcali, Adana 01330, TURKEY; and Mete K. Gulmen, PhD, MD, Cukurova University, School of Medicine, Dept of Forensic Medicine, Adana 01330, TURKEY*

After attending this presentation, attendees will better understand the effect of the number of STR loci to the kinship analysis.

This presentation will impact the forensic science community by illustrating how the number of STR loci could be critical for the evaluation of relatives, especially close relatives.

In this presentation, discrimination power will be calculated with allele frequency distribution in nine STR loci of people who live in the Cukurova region of Turkey and the statistical significance for forensic identification and kinship analysis will be detected. The results obtained from this study will be used as a database to calculate the match probability of the individualization and kinship analysis in a statistical evaluation in a Cukurova population.

STRs are the structures with two to six base pairs in length that repeat like the heads and tails in the human genome.[1] Since the number of these structures differ among individuals, they are used in forensic individualization and kinship analysis.[2] Allele frequencies of STR loci show differences among populations. Therefore, before the use of STR loci in individualization and kinship analysis, allele frequency distribution related to the population of that locus must be determined. In an attempt to specify identification and kinship, analyses of 15 STR loci are routinely made; however, when the population number in a DNA database increases more and more in time, these standard STR loci that are determined in studies, especially between close relatives, may become inadequate.[3] In such circumstances, the usage of different STR loci in addition to this set could increase discrimination power and may possibly make forensic cases more accurate and qualified.

For the United States national database, 13 STR loci were determined as Combined DNA Index System (CODIS) markers in 1997.[4,5] With later studies in 2001, a commercial kit named Identifiler®, which consists of 15 STR loci, was produced.[5] Next, the VeriFiler™ kit, which consists of additional STR loci, was released as a commercial kit in 2013. Both commercial kits share three autosomal STR loci (D2S1338, D19S433, and THO1) and sex typing locus Amelogenin (AMEL) as internal genes.[6,7] In circumstances where the discrimination power is inadequate, additional STR loci in addition to 15 STR loci are studied.

In this study, genomic DNA samples prepared from whole blood and oral swabs taken from 100 unrelated healthy volunteers who applied for kinship analysis to the Cukurova University Department of Forensic Medicine were used to determine the allele frequencies of nine STR loci. The analysis of nine autosomal STR loci (D6S1043, D1S1656, D2S441, D10S1248, D12S391, D22S1045, THO1, D2S1338, and D19S433) and the sex typing marker AMEL within a commercial kit in addition to 15 STR loci within another commercial kit used in routine forensic cases was performed. The allele frequency 0.3 for D10S1248 with allele 14, 0.17 for D1S1656 with allele 16, 0.185 for D2S1338 with allele 17, 0.415 for D22S1045 with allele 15, 0.315 for D19S433 with allele 14, 0.25 for THO1 with allele 6, 0.345 for D2S441 with allele 11, 0.275 for D6S1043 with allele 12, and 0.19 for D12S391 with allele 19 were identified as being the most frequent. Combined power of discrimination was found to be 0.9998.

In routine cases, the paternity index remains below 10,000 with 15 STR loci. It was observed that the paternity index could be increased to more than 10,000 by an additional six new STR loci.

In conclusion, this study shows that additional STR loci provides accurate and qualified results to identify people when the discrimination power is inadequate. With this study, allele frequencies of six new (three over again) STR loci were determined in a Cukurova population for the first time.

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Allele Frequency, Cukurova Population, STR Analysis

H16 Interactions Between Microbes and Larvae on a Human Corpse

Vadim Mesli, MD, Institut Medico Legal, Rue Andre Verhaeghe, CHRU Lille, Lille Cedex, Nord 59037, FRANCE; Damien Charabidze, PhD, Univ Lille 2, Rue A. Verhaeghe, Lille 59000, FRANCE; Valéry C. Hedouin, MD, PhD, Iml-chu Lille, Rue Andre Verraeghe, Lille 59000, FRANCE; Christel Neut, PhD, Laboratoire de Bactériologie Clinique, 3 rue du Professeur Laguesse, Lille 59006, FRANCE; and Didier Gosset, MD, PhD, Institut de Medecine Legale, Faculte de Medecine, Lille 59045, FRANCE*

After attending this presentation, attendees will better understand the interactions between bacteria and insects that are present on the skin of a dead human body.

This presentation will impact the forensic science community by providing information on the two major entities (bacteria and insects) in corpse decomposition, which can be used in the postmortem interval evaluation.

After death, bacteria within the cadaver play an important role in its decomposition. At the same time, when necrophageous insects are present, they actively participate in the body degradation. The postmortem interval determination can be based on the study of necrophageous insects on the body, and studies of the cadaver's microbiota had shown promising results in this area of research. Interactions between specific bacteria and Calliphoridae larvae have been described in studies about maggot debridement therapy, but there are few data on interactions between those two entities in a forensic approach. This study seeks to specify the interactions between bacteria and Calliphoridae larvae, which are found on the skin surface of dead human bodies.

Methods: Two different protocols were performed. The first consisted of sampling larvae on human corpses and extracting their Excretion/Secretion (ES) fluids. Bacterial isolation and identification were performed on the ES using cultural methods, completed by mass spectroscopy for identification. During the second protocol, sterile *Lucilia sericata* larvae were confronted with three bacterial strains — *Wohlfahrtiimonas chitiniclastica*, *Providencia rettgeri*, and *Proteus vulgaris* — (selected in the first protocol) in monobacterial-inoculated petri dishes. Statistical tests were performed on the size and survival of the larvae.

Results: ES fluids were sampled from two different bodies (these experiments are still in progress), with polymicrobial contamination associating bacteria from the intestinal microbiota, the larval microbiota, or the environment. Concerning the interactions between bacteria and larvae, significant differences (KW=11,226, p=0,009) were observed for the larval average sizes depending on their confrontation to different bacterial strains and the control.

Discussion: The bacterial genera or species identified in the ES of larvae sampled on the skin came primarily from intestinal microbiota; however, the association with bacteria coming from the environment or the larval microbiota is in favor of the existence of ecosystems localized in specific areas of the cadaver. Data gained from this research will allow a better appreciation of the human corpse microbiota when colonized by insects, as well as their interactions and could be taken into account when interpreting postmortem interval, which is a major issue in forensic investigations.

Taphonomy, Postmortem Microbiology, Forensic Entomology

H17 Can DNA Data Be Used to Establish a Cut-Off Time for Juvenile Sexual Assault Exams?

Daniela Anane-Bediakoh, BS, 142 Zia Dodda Crescent, Brampton, ON L6P1N3, CANADA; Martin Lopez, MS, Houston Police Department, 1202 Washington Avenue, Houston, TX 77002; Holly Whillock, BS, Houston Police Department, 1200 Travis Street, Houston, TX 77002; Sheree R. Hughes-Stamm, PhD, Sam Houston State University, Dept of Forensic Science, Huntsville, TX 77341; and Amy Castillo, PhD, 1200 Travis Street, 20th Fl, Houston, TX 77002*

After attending this presentation, attendees will better understand the importance of prompt evidence collection in juvenile sexual assault cases, the link between DNA quantification results and downstream Short Tandem Repeat (STR) success, potential trauma caused by sexual assault exams, and the necessity in developing a post-incident cut-off time.

This presentation will impact the forensic science community by providing guidance on how to better serve juvenile sexual assault victims by helping to determine when an invasive, potentially traumatic, sexual assault examination improves an investigation. This research will help inform the forensic science community as to when the examination maximizes the likelihood of developing a profile while inflicting the least amount of trauma on the juvenile.

The knowledge gained from a sexual assault exam can be beneficial in the investigation of an alleged juvenile sexual assault. Nevertheless, it is critical to balance the information's value with the victim's trauma. A Sexual Assault Nurse Examiner (SANE) collects evidence from the victim as part of a comprehensive exam. The resulting Sexual Assault Kit is then processed to determine if a DNA profile foreign to the victim is present.

To have optimal effect, the Sexual Assault Exam should be done a short time after the alleged assault. While this is common knowledge, there is often a time lapse between the sexual assault of a juvenile and the Sexual Assault Exam. This often results either from a delayed outcry or because parents are unaware of the assault. Sexual assault exams may cause secondary trauma to the victim, in addition to the significant, initial trauma caused by the sexual assault. Previous publications have suggested that the Sexual Assault Exam be conducted within 24 hours in pre-pubescent children and within 72 hours in adolescents. Therefore, it should be considered that the trauma caused by the Sexual Assault Exam may not yield any evidence when collected outside of these published guidelines. Therefore, it is important to gain a better understanding of the relationship between the time interval between assault and collection and the downstream DNA profiling success.

This study will present the results of an extensive review of more than 1,000 sexual assault cases involving juveniles. The review focuses on DNA quantification data, the ratio between the amount of autosomal and male DNA detected and the success of retrieving a useable DNA profile foreign to the victim. The sooner SANEs are able to collect samples from the juveniles, the more likely it is that a profile will be developed that is Combined DNA Index System (CODIS) eligible. This study endeavors to determine the optimal time interval for the collection of a sexual assault kit based on the success of downstream DNA profiling results for the possible offender. In addition, this data may be used to develop a cut-off time post incident. This would allow SANEs to decline a sexual assault examination on juvenile victims based on the premise that the risk of inflicting further trauma may outweigh the likelihood of gaining a useable DNA profile.

Juvenile, Sexual Assault, DNA

H18 Application of a 6-Plex Microsatellite Kit in the Analysis of Aged Fecal DNA Samples: Prospective Use in Equine Slaughter Forensic Cases

Ketaki Deshpande, MS, 10000 NW 9th Street Circle, Apt 9, Miami, FL 33172; Melissa V. Oswald, MSFS, Florida International University, 11200 SW 8th Street, Miami, FL 33199; and DeEtta Mills, PhD, Florida International University, OE 167, Biological Sciences, 11200 SW 8th Street, Miami, FL 33199*

The goal of this presentation is to demonstrate to the animal forensics community an effective DNA extraction and isolation technique using non-invasive fecal sampling.

This presentation will impact the forensic science community by adding to research being carried out in animal and wildlife forensics by broadening the available tools for DNA isolation in cases of degraded biological samples and fecal samples with unknown days since defecation.

Feces represent an unlimited and easily available source of DNA that can be used in forensic cases of domestic animals or wildlife. In cases of equine slaughter, fecal samples from stolen or missing horses can be used to identify and match to the remains of a slaughtered horse. In studies of elusive or endangered species, the advantage of fecal analysis is non-invasive sample collection, allowing more frequent sampling of individuals without having to capture the animals; however, quite often amplification of DNA from extracted feces is compromised by environmental contaminants and dietary inhibitors coupled with low quantity and poor quality of genomic DNA. In the present study, non-invasive sampling of fecal matter from ten domestic horses was used to develop the methods when fecal samples were aged up to six days from each individual. Genotypes were known for all horses. Field validation of five additional samples was conducted when fecal donors and days since defecation were unknown. A viable protocol for fecal DNA extraction and efficient genotyping using a 6-plex (VHL20, HTG4, HTG6, HMS7, HTG7, and HMS3) of equine microsatellite markers was demonstrated.

Methods: The extraction technique included using a modified QIAGEN® QIAmp® DNA Stool Mini Kit protocol coupled with Pressure Cycling Technology (PCT). The modification to the manufacturer's protocol and incorporation of PCT when hydrostatic pressure was used in the lysis of cells ensured maximal DNA output and clean up from inhibitors.

Results: This technique yielded complete (six loci) equine DNA profiles for 80% of the samples ≤ two days old and 40% of samples after six days of aging. Kinship for the ten domestic horses and the "unknown" field samples based on the six loci using the ML-Relate software was also determined.

Conclusion: PCT along with the modified extraction method increased the likelihood of obtaining an equine DNA profile from fecal samples. This study provided a technique for degraded and compromised DNA evidence that can be used to identify individuals in animal forensic cases and equine slaughter cases when fecal samples may be the only evidence available.

Fecal Matter, Pressure Cycling Technology, Equine

H19 The Assessment of GeoChip™ Functional Gene Microarray as an Aid for Soil Provenance

Priyanka Kushwaha, MS, Florida International University, 11200 SW 8th Street, Miami, FL 33199; and DeEtta Mills, PhD, Florida International University, OE 167, Biological Sciences, 11200 SW 8th Street, Miami, FL 33199*

The goal of this presentation is to demonstrate the use of the functional gene microarray GeoChip™ 5.0. This array can be used for discrimination and provenance of soil samples. This technique is a useful platform to study microbial communities and how their functional guild associations can strengthen the forensic investigations of soil.

This presentation will impact the forensic science community by demonstrating the utility of functional gene analyses to refine the use of microbial community profiles for soil provenance. The GeoChip™ microarrays are rapid, robust, and can analyze approximately 180,000 functional genes with known taxonomic affiliations.

Soils have served as an important evidence in forensic investigations owing to their mineral and organic properties.[1] Nevertheless, the physical examination of soils does not always result in successful categorization of the samples. Therefore, complementary approaches such as DNA profiling of soil bacterial communities using Terminal Restriction Fragment Length Polymorphism (T-RFLP) for forensic comparison and discrimination of soils have been done in the past.[2] Although soil microbial profiles characterized using 16S ribosomal RNA (rRNA) hypervariable domains have been successful in discrimination of soil samples, the 16S rRNA domain is not adequate to establish microbial functional diversity.[3] As soil microbial community plays an important role in maintaining the fertility and stability of soils by cycling the biochemical nutrients, it is imperative to understand the function of these microbial communities and add those data to existing methods to refine the ability to discriminate between soil samples.[4]

GeoChip™ is a functional microarray that contains oligonucleotides probes for genes involved in all of the biogeochemical cycles, stress-related genes, *gyrB*-based phylogenetic markers, antibiotic resistance genes, and many others. Hence, it can detect thousands of microbial functional genes and phylogenetic markers at the same time.[5,6] The objective of the study was to compare the functional gene profiles of bacterial, archaeal, fungal, protists, and viral communities between two different soil types: Lauderhill Dania-Pahokee (Soil type 2; represented as Krome North Tower (KNT) transect) and Perrine-Biscayne-Pennsuco (Soil type 4; represented as Card Sound (CS) transect) of Miami-Dade County, FL.

DNA was extracted from soil samples (n=15) collected from one transect belonging to each soil type. DNA was precipitated with 100% ethanol and 0.3M NaOAc and DNA purity was assessed using Ultraviolet (UV) absorbance, dried and shipped to the Institute of Environmental Genomics (IEG), University of Oklahoma, Norman, OK, for processing the GeoChip™ 5.0 microarray. The raw data was pre-processed using the IEG data analysis pipeline and Principal Component Analysis (PCA) on carbon and sulfur cycle genes was performed.

PCA analysis of carbon and sulfur cycle genes resulted in clustering of the two soil samples onto different principle components. In addition, unpaired Student's t-test revealed the genes (*AceB*, *CsoS1_CcmK*, *CsoS2*, *FBP_aldolase*, *FBPase*, *GAPDH_Calvin*, *PRI*, and *TIM*) representing the carbon cycle and sulfur cycle genes (*APS_APrB*, *APS_kinase_protist*, *Sir*, *dsrB*, *dsra*, *soxA*, and *soxB*) that were significantly different ($p < 0.05$) for KNT and CS. In conclusion, this study was able to discriminate these different soil types from each other. The assessment of both the phylogenetic and functional genes of soil communities together will assist in higher discrimination of soil samples for provenance and forensic applications.

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GeoChip™ Microarray, Functional Genes, Microbial Community

H20 Comparison of Extraction Methods From Cotton Swabs in Reference to Background DNA From Commonly Touched Surfaces

Meghan Roig, BS, Florida International University, 11200 SW 8th Street, Miami, FL 33199; Thais Simoes, Florida International University, 11200 SW 8th Street, Miami, FL 33199; Bruce R. McCord, PhD, Florida International University, Dept of Chemistry, University Park, Miami, FL 33199; and Kerry Lynn Opel, MA, PhD, Upper Iowa University, PO Box 1857, Fayette, IA 52142*

After attending this presentation, attendees will have a greater understanding of background touch DNA, the variation in quantities of DNA on different surfaces over time, and methodology for DNA extraction from cotton swabs.

This presentation will impact the forensic science community by providing information regarding levels of background DNA as well as by discussing swabbing and extraction methods. This presentation will improve understanding of the levels of background DNA on touched objects as well as demonstrate a novel extraction method.

With every touch of the skin to a surface, cells are left behind; with every cell, the genetic code can be found. People touch doors, tables, and so many other surfaces every day, often with multiple people touching the same object through the course of a day.[1] When a swab is used for evidence collection from a surface, the investigator may not know how many people may have touched this evidence in the past or what level of persistence DNA may have on touched objects over time. Though every touch leaves cells containing DNA, most contact leaves only a few cells if minimal pressure is used. These trace levels of DNA may remain undetected or, if detected, may be at such low levels that only stochastic effects and low levels of allele drop-in are seen.[2] Thus, it is important to understand general levels of DNA on commonly touched objects. To complete this properly, it is important to optimize extraction efficiency and to maximize DNA recovery from swabs. Therefore, levels of DNA on commonly touched objects are being investigating.

In this project, various surfaces were swabbed with a damp cotton swab, including bathroom door handles, benches, public areas, and household items. The DNA was extracted using standard phenol chloroform extraction along with alkaline lysis and Pressure Cycling Technology (PCT). A variety of different conditions were examined and optimized. For the PCT extractions, temperature, pressure, and time were investigated to increase the quantity of DNA resulting from the touched surfaces.[3] Alkaline lysis methods were also examined to improve recovery of DNA from swabs. This procedure was optimized by determining the effect of time and temperature on the recovered DNA. The quantity of recovered DNA was determined using real-time Polymerase Chain Reaction (PCR) with *Alu*-based targets and SYBR green detection. The samples were also analyzed using capillary electrophoresis-based Short Tandem Repeat (STR) typing to determine the percentage of recoverable alleles.

Touch DNA is an emulation of the Locard exchange principle, in that any time a person is in a location, that person may leave evidence of their presence.[1] The key issues are what levels of DNA can be found on everyday touched objects and how long do such levels of DNA persist following the incident. Results will be shown from a variety of surfaces found in public and private areas.

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Background Touch DNA, Pressure Cycling Technology, Low Copy DNA

H21 Wildlife Forensic Investigation: Identifying an Unidentified Specimen Using NADH Subunit 2 (*ND2*) and Cytochrome B (*cytb*) Genes

Hailey Mcclenon, 2906 Palm Street, Apt 1, Houston, TX 77004; Ashraf Mozayani, PharmD, PhD, Texas Southern University, 3100 Cleburne Avenue, Houston, TX 77004; and Hector Miranda, PhD, Texas Southern University, 3100 Cleburne Street, Houston, TX 77004*

After attending this presentation, attendees will understand the principles in determining the true identity of an unidentified carcass donated by the Houston Museum of Natural History; the use of DNA markers as an indispensable tool in the practice of wildlife forensics and monitoring of endangered species; and the elements for the application of mitochondrial gene markers *cytb* and *ND2* in wildlife crimes.

This presentation will impact the forensic science community by explaining the application of two commonly used mitochondrial DNA markers to establish a true DNA profile and create a personalized barcode based upon the species' closest relatives as a reference to the animal's geographic origin which, when applied, could potentially decrease the current amount of slaughter and harvest involved in illegal trade routes within the black market.

The unidentified species sample was received by the Department of Biology at Texas Southern University. To determine the fidelity of DNA markers to identify the exact wildlife species beyond doubt, two mitochondrial gene markers, cytb and ND2, were used. DNeasy Tissue Kit was used to isolate genomic DNA. *Cytb* and *ND2* were sequenced using primer pairs L14990/H15646, L15517/H16404 for *cytb* and L5216/H5766, L5758/H6313 for *ND2*. Polymerase Chain Reaction (PCR) was carried out in a total volume of 25µL containing 10ng-50ng of template DNA, 4µL of deionized water, 4µL 10mM of each dNTP, and 8µL of AmpliTaq® 360 Gold Polymerase Master Mix. The PCR cycles were as follows: one cycle of 10m at 95°C, 35 cycles of 20s at 95°C, 20s at 60°C, and 50s at 72°C. The process was completed with a final elongation at 72°C for 10m. These parameters were adjusted to optimize PCR product quality. Amplifications were performed on Veriti® Thermal Cycler. The PCR products were about 1,000bp for *ND2* and 1,100bp for *cytb* gene. The PCR products were sequenced using ABI® 3730, then viewed and examined in Geneious Pro. Basic Local Alignment Search Tool (BLAST) was used to compare DNA sequences in Genbank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The results suggested the specimen was a Malayan Peacock-Pheasant (Polyplectron malacense) with 100% identities for ND2 and cytb genes. This species is classified as vulnerable on the International Union for the conservation of Nature Red List. This approach of using two, instead of just one, gene marker for wildlife forensics can greatly contribute to the confidence in wildlife monitoring and trafficking.

Wildlife Forensics, NADH Subunit 2 (*ND2*), Cytochrome B (*cytb*)

H22 Forensic Taphonomy: Investigating the Relationship Between Gross Postmortem Change and Mass Loss

Adam Orimoto, MS, Honolulu Police Department, 801 S Beretania Street, Honolulu, HI 96813; Kanani N. Thompson, 1037 Noble Lane, Apt A, Honolulu, HI 96817; Emily Junkins, BS, Chaminade University of Honolulu Forensic Sciences, 3140 Waialae Avenue, Honolulu, HI 96816; Christopher G. Inoue, BS, Honolulu Department of the ME, 835 Iwilei Road, Honolulu, HI 96817; and David O. Carter, PhD, Chaminade University of Honolulu, Forensic Sciences Unit, Honolulu, HI 96816*

After attending this presentation, attendees will understand that there is a significant positive correlation between gross postmortem change and carcass mass loss.

This presentation will impact the forensic science community by allowing investigators to accurately estimate mass loss, which will facilitate the estimation of postmortem interval as well as providing a dataset that is foundational to the development of an objective method to estimate carcass decomposition.

One of the most challenging components of a death investigation is estimating the Postmortem Interval (PMI). This is most likely due to corpse decomposition being poorly understood. Typically, death investigators estimate the PMI based on non-standardized methods such as anecdotal evidence and visual judgment based on mass loss. Though these methods provide valuable and needed information for a death investigator, many factors such as seasons, climates, and differences in perception skew the estimation of PMI. The search for a standardized way to estimate PMI across differing death scenes remains unresolved. Recently, a decomposition scoring system (Total Body Score (TBS)) was established to visually describe and document the progress of decomposition; however, it remains to be determined if TBS, an indirect measure of decomposition, is correlated to actual mass loss, a direct measure of decomposition. This is important to determine because a newly established method to estimate PMI requires an estimate of soft tissue mass loss, the primary variable in the visual estimation of PMI. If it can be determined that mass loss and TBS are correlated, then it would be possible to estimate soft tissue mass loss more effectively. This, in turn, will allow criminal investigators to estimate PMI more effectively.

To address this gap in knowledge, a 17-day experiment was conducted to measure mass loss and its relationship to TBS. Three swine carcasses (*Sus scrofa domesticus*) were placed in a field setting in Palolo Valley, Oahu, HI. Carcasses were killed by means of electrocution and placed at the site one hour postmortem. Carcasses were weighed (kg) and TBS was measured twice a day for 13 days and once a day every other day for the remainder of the experiment.

Using Spearman's Rank Correlation, a significant ($P < 0.0001$, $R^2 = 0.962$) positive relationship between mass loss and TBS was observed. This relationship was described with a fourth order polynomial equation ($y = -0.0008x^4 + 0.049x^3 + 0.78x^2 + 5.4x - 10.88$) where y=soft tissue mass loss (%) and x=TBS. Thus, the fact that TBS can be used to accurately estimate mass loss is concluded. In practice, an investigator would calculate TBS as x, then substitute x for TBS in the above equation. For example, a TBS of 14 would equate to a mass loss of approximately 17%. It is expected that this approach will aid criminal investigators in estimating postmortem interval more effectively by allowing crime scene investigators to generate a quick and accurate estimation of PMI based on the calculated mass loss percentage and the TBS.

The current results show that gross decomposition has a direct relationship to the mass loss of a carcass. In other words, the physical characteristics of a corpse can provide insight into the amount of decomposition that has occurred. This study is important because it contributes to a limited dataset of direct decomposition measurements. This dataset provides a quantitative assessment of taphonomy on Oahu, HI. It is important to consider that the patterns observed in this experiment are specific to the tropical climate in which they were conducted. This study also does not take into account how decomposition patterns can change based on antemortem wounds. With refining, this study could be used by investigators to identify gross postmortem change and estimate mass loss.

Postmortem Interval, Decomposition, Death Investigation

H23 Dynamics of Decomposition in Tropical Environments: A Multidisciplinary Approach

Ashley A. Matchett, InterAmerican University of PR-Bayamon Campus, 500 Dr John Will Harris Drive, Bayamon, PR 00957; and Jariangely Rivera, Cond. Vizcaya Aprt. 7-14 #200 C/535, Carolina, PR*

After attending this presentation, attendees will understand how climate variation can affect the entomological and microbiological phases of the decomposition of a pig in a tropical environment with the observation of the taphonomy.

This presentation will impact the forensic science community by providing the results of a multidisciplinary experiment that unifies different fields of science in a tropical environment, which has not been previously accomplished. The goal of this investigation is to contribute to the identification of the postmortem interval through the taphonomy of the organism utilized in this study — the interaction and presence of the bacteria in the pig and in the insects in every phase of the decomposition. This will provide a better understanding about the identification of the phase or time of an organism after the subject's death.

In forensic death investigations, establishing the Postmortem Interval (PMI) of the human cadavers is important for the forensic environment, for example, in reconstructing the scene and approximate time in which the events took place. This is particularly important in a tropical environment where the decomposition processes are accelerated due to heat and humidity; however, a shortcoming is the correlation between the different PMI estimates from independent monitoring techniques. The objective of this investigation is to determine the PMI by integration of a number of recently developed techniques in PMI estimation for a correlative multidisciplinary approach. For reproducibility, standardization, and ease of handling, a model system of a domestic pig (*Sus scrofa*) was used to monitor decomposition. This study is part of an ongoing study of decomposition in Puerto Rico to establish correlations and differences between the multiple environments found in the Atlantic tropical cyclonic climate. The environment used in this particular study was a naturally protected wetland area in Puerto Rico. Sample collection was undertaken in duplicate, along with data, of three daily intervals with continuous environmental and climate monitoring.

Climate monitoring was undertaken in order to normalize and access its relationship to decomposition via a nearby meteorological station and included measurements of predominate wind directions, pressure, rainfall, temperature, and humidity. In addition, the cadaver was locally monitored for external and internal temperature variation along with the surrounding soil. In order to assess biological and microbiological contributions or changes, analysis of the surrounding soil was conducted before, during, and after decomposition.

The decomposition rate and changes, including entomological population dynamics, were monitored by high-resolution photography throughout the decomposition process. Total entomological population estimates were calculated along with sampling for morphological and genetic identification to determine the nature of entomological decomposition dynamics.

Microbial decomposition dynamics was assessed by quantitative microbial culture, biochemical identification, and Next Generation 16S ribosomal RNA (rRNA) metagenomics of the samples in multiple locations of the cadaver, notably the skin in multiple locations and the oral, nasal, and anal cavities.

The decomposition of the cadaver to skeletal elements in the tropical environment took 25 days, during which the five stages of the decomposition were determined. The "Initial Decay" lasted five hours and fourteen minutes in which the arrival of the first carrion flies (*Calliphora*) and three crickets (*Gryllidae*) were observed. The following stage, "Putrefaction," lasted from the same night until day 3. The presence of blue flies (*Calliphora*), black flies (*Phormia*), and green flies (*Lucilia*) increased in day 3, where up to 38 flies were found on the cadaver; additionally, the presence of ants and the initial hatching of larvae started on day 2. The "Black Putrefaction" stage started on the morning of day 4 and ended on the evening of day 14; by day 5 the only flies present were the black species and by day 6 a large number of larva were in the process of becoming pulps. The advanced putrefaction or "Butyric Fermentation" stage commenced on day 15 and ended on day 21 as the larvae started to leave the cadaver; it was observed that there was little entomological activity. By day 20, the cadaver was in "Dry Decay," was partially skeletonized, and the advanced putrefaction had ended since the majority of the bones were visible.

From this preliminary investigation, a greater understanding of the dynamics of decomposition in tropical environments has been deduced and some correlations have been made by using a variety of techniques including the population and species variation of both microbes and insects with appropriate controls, although the variation and high rate of decomposition together with local effects require further study for a clearer understanding of the inter-relationships between the local environment, flora, and microbiota; however, the strength of this approach is evident and could allow for an increased precision in determining the PMI through multiple and independent assessment techniques.

PMI, Descomposition, Tropical

H24 Severe Retinal Hemorrhages With Retinoschisis Are Not Pathognomonic for Abusive Head Trauma

Kenneth D. Hutchins, MD, Miami-Dade County, Medical Examiner Dept, Number One on Bob Hope Road, Miami, FL 33136; and Mark J. Shuman, MD, Miami-Dade County, ME Dept, Number One on Bob Hope Road, Miami, FL 33136*

After attending this presentation, attendees will learn that severe retinal hemorrhages with retinoschisis can occur in infants and toddlers following head injury from short falls and from spontaneous intracranial hemorrhage due to ruptured, dural-based vascular malformations.

This presentation will impact the forensic science community by increasing the attendees' awareness of the natural and traumatic conditions associated with severe retinal hemorrhages and retinoschisis in infants and toddlers.

The combination of subdural hemorrhage, retinal hemorrhage, and encephalopathy, or the presence of severe retinal hemorrhages, alone in infants is often stated to be pathognomonic for abusive head trauma; however, the same constellation of findings has been identified in accidental head injuries and natural diseases. Presented here are two cases of severe retinal hemorrhages with retinoschisis associated with subdural hemorrhage from a ruptured vascular malformation and with severe cerebral edema in an accidental head injury.

Case Report 1: A 3½-month-old infant suddenly began making choking noises, arched his back, stiffened, and then became lethargic. He was driven to a hospital where he was found to be lethargic (GCS 9), and had flat fontanelles and equal, round, and reactive pupils. A Computed Tomography (CT) scan of his head revealed "a large left-sided interhemispheric subdural hematoma" that extended over the left frontoparietal convexity and caused approximately 4mm of midline shift. He was transported to a tertiary hospital with a Pediatric Intensive Care Unit (PICU). His condition deteriorated and a repeat CT scan revealed worsening of the hemorrhage and mass effect. He underwent an emergency craniectomy and hematoma evacuation. An ophthalmology examination two days later revealed pre-retinal hemorrhage with areas of schisis in the right eye and possible vitreous hemorrhage in the left eye. The findings were said to be "consistent with non-accidental trauma." He expired the evening of the fourth hospital day. The clinical diagnosis was non-accidental head injury.

The autopsy revealed a well-developed, anasarcous, infant male who was status post left frontal-temporal-parietal craniectomy and had a large, predominantly left-sided subdural hemorrhage, extensive intradural hemorrhage of the falx cerebri, subarachnoid hemorrhage, right frontal and occipital lobe infarcts, cerebral edema, bilateral optic nerve sheath hemorrhages, and extensive bilateral retinal hemorrhages and retinoschisis. A vascular malformation of the falx cerebri was identified and confirmed with immunohistochemistry for CD31 and CD34. The cause of death was a ruptured vascular malformation of the falx cerebri and the manner of death was natural.

Case Report 2: A 14-month-old toddler was on a train ride in a mall when he stood on the seat, fell, and struck his head on the ground. He was unresponsive, bleeding from his head, bradycardic, and his pupils were non-reactive. He was intubated and airlifted to a hospital. A CT scan of his head revealed diffuse cerebral edema, loss of the gray/white matter interface, diffuse subarachnoid hemorrhage, and a left occipital fracture. He progressed to brain death approximately one week later.

The autopsy revealed an abrasion of his face, scalp and subgaleal hemorrhage, a left occipital skull fracture, diffuse subarachnoid hemorrhage, cerebral edema, necrosis of the cervical spinal cord, subdural hemorrhage of the spinal cord, and bilateral optic nerve sheath and retinal hemorrhages with a circinate retinal fold in the left eye. The cause of death was blunt head injury and the manner of death was accident.

The presence of severe retinal hemorrhages with retinoschisis in young children is often said to be pathognomonic of abusive head trauma and this was the clinical diagnosis in Case 1. The interhemispheric location of the large subdural hemorrhage in Case 1 and the extensive hemorrhage in the falx cerebri found at autopsy are not typical of trauma, and a vascular malformation in the falx cerebri was identified as the cause of the bleeding. This child had severe, bilateral retinal hemorrhages with bilateral retinoschisis and had a clinical diagnosis of abusive head trauma. Case 2 was a clearly documented and witnessed accidental fall, which resulted in an occipital skull fracture, diffuse subarachnoid hemorrhage, cerebral edema, and bilateral retinal hemorrhages with left retinoschisis. These cases illustrate that the presence of severe retinal hemorrhages with retinoschisis are not pathognomonic for abusive head trauma.

Retinal Hemorrhage, Retinoschisis, Abusive Head Trauma

H25 Evaluation of the Presence and Distribution of Leptomeningeal Inflammation in Cases of Sudden Death in Infancy

Esther Jack, MB BCH BAO, Office of the State Pathologist, C/O Fire Brigade Training Centre, Malahide Road, Marino Dublin 3, IRELAND; Elisabeth Haas, MPH, Rady Children's Hospital, Dept of Pathology, 3020 Children's Way, MC--5007, San Diego, CA 92123; and Terri L. Haddix, MD, PO Box 394, Corte Madera, CA 94976*

After attending this presentation, attendees will better understand the relevance of the presence of inflammatory cells and iron in the leptomeninges of infant brains.

This presentation will impact the forensic science community by providing a baseline for comparison of neuropathologic findings in infant brains, specifically the degree of inflammatory infiltrates and iron in the leptomeninges of infants with no evidence of trauma and generally covered by the Sudden Infant Death Syndrome/Sudden Unexpected Death in Infancy (SIDS/ SUDI) designation.

Prior research has demonstrated that the leptomeninges of infants and late-term fetuses derived from a hospital-based (non-traumatic) cohort contain a surprisingly large number of inflammatory cells and stainable iron. These observations were present irrespective of the findings from the general autopsy and neuropathologic examination and irrespective of the mode of delivery. A similar methodology was sought to a more forensically relevant population, specifically infants whose deaths are broadly classified as SIDS/ SUDI.

Forty-two SIDS/SUDI cases autopsied between 2006-2014 by the San Diego County Medical Examiner's Office were identified. An interpretable amount of leptomeninges from one to three areas of the brain (cerebral cortex, brain stem, and/or cerebellum) in each case were evaluated. Immunoperoxidase staining of each of these sections with antibodies to CD45 and CD68 were performed. Additionally, each section was evaluated for the presence of iron utilizing Perl's method. Each slide was screened manually, immunoreactive cells were individually scored, and the density was calculated per millimeter of leptomeninges. The presence of stainable iron was similarly scored.

The cohort represented 22 males and 20 females ranging in age from 2 to 311 days. The modes of delivery were relatively evenly divided. The reported causes of death were SIDS/SUDI (62%), SIDS with bed-sharing (36%), and undetermined (2%). The assigned manners of death were 62% natural and 38% undetermined. The examined brain sections included 32 of the cerebral cortex, 18 of the brain stem, and 36 of the cerebellum. The lengths of examined leptomeninges ranged from 2mm to 40mm. In the total cohort, the mean number of CD45 and CD68 immunoreactive cells in the cerebral cortical sections was 7.5 cells/mm and 22.1 cells/mm, respectively; in the brain stem sections, 10.8 cells/mm and 16.7 cells/mm, respectively; and in the cerebellar sections, 9.9 cells/mm and 27.5 cells/mm, respectively. The ranges of the number of cells per mm, and the standard deviations of the means, were wide and varied. Overall, there was no significant difference in the number of CD45 or CD68 immunoreactive cells/mm between the three brain sites. Comparing this cohort to a subpopulation of hospitalized infants in the prior study, there were no significant differences between the density of inflammatory cells in the sections from the cerebral cortex and brain stem. There were differences in the CD68 density in the cerebellar sections which are attributable to methodological differences; however, there was a significant difference in the number of iron-containing cells between the two populations with iron identified in only a single section in the current cohort but in 30 sections in the corresponding hospital-based cohort.

Whether in a hospital-based or more forensically relevant population, the presence of inflammatory cells in the leptomeninges (even in great abundance) is common. Thus, caution must be exercised in the interpretation of this inflammation and its clinical/diagnostic relevance. The finding of iron deposition in the leptomeninges, though, is an extremely uncommon finding in the SIDS/SUDI population, which is at odds with the prior findings in a hospital-based population.

Leptomeninges, Inflammation, Infant

H26 Nerve Root and Dorsal Root Ganglia (NR/DRG) Hemorrhage as an Indicator of Pediatric Traumatic Head Injury (THI)

Marianne E. Beynon, MD, Baylor College of Medicine, Dept of Pathology & Immunology, 1 Baylor Plaza, BCM 315, Houston, TX 77030; Miriam E. Soto Martinez, MA, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; Jo Elle G. Peterson, 940 Stanton L Young Boulevard, BMSB 451, Oklahoma City, OK 73104; Jennifer C. Love, PhD, OCME, 401 E Street, SW, Washington, DC 20024; Dwayne A. Wolf, MD, PhD, Harris County ME, JAJ Forensic Center, 1885 Old Spanish Trail, Houston, TX 77054; and Glenn D. Sandberg, MD, Harris Co Inst of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will better understand a standardized, concise methodology for evaluating and reporting NR/DRG hemorrhage and will be knowledgeable regarding the comparison between NR/DRG hemorrhage in traumatic and non-traumatic pediatric deaths.

This presentation will impact the forensic science community by providing a powerful technique to assist in distinguishing between pediatric decedents with and without significant THI. Additionally, this study shows the importance of careful spinal cord examination in cases of suspected pediatric head trauma.

An association between pediatric THI and hemorrhage within the cervical NR/DRG was first described by Downs in 2005.[1] Matshes et al. suggest that these injuries are secondary to forces transferred to the spinal cord and adjacent NR/DRG during hyperflexion-extension of the relatively weak infant neck; the resulting damage to the C3-5 nerve roots would cause diaphragmatic paralysis and subsequent fatal anoxic brain injury.[2] Cervical nerve root injury in fatal THI was also reported by Brennan et al. and Sens et al.[3,4] In 2014, the Harris County Institute of Forensic Sciences (HCIFS) completed a small pilot study which showed correlation between cervical NR/DRG hemorrhage and THI in a pediatric population, supporting previous studies.[5,6]

To further investigate the relationship between NR/DRG hemorrhage and pediatric death, a nine-month prospective study was conducted. All infants autopsied by HCIFS meeting the age criterion (0-12 months old at time of death) were included, except for individuals with survival time greater than one week after terminal hospital admission. The spinal cord with attached NR/DRG was removed in each case via modified posterior approach as described by Peterson et al.[5,6] The tissue was fixed for two weeks in 20% formalin, then sectioned and Hematoxylin-Eosin (H&E) stained following standard methods. Each section was examined by a staff neuropathologist and a pathology resident who were both blinded to cause and manner of death.

To standardize examination, a scoring method was developed: presence of hemorrhage in each individual NR/DRG was scored on a scale of 0 to 2 (0=no hemorrhage, 1=scant hemorrhage, 2=prominent hemorrhage). Twenty-five cases were scored by two individuals to assess inter-rater agreement using the scoring methodology. A moderate level of agreement was identified between raters in all compared parameters (Cohen's Kappa=0.43). Of note, all disagreements occurred between the scores of 0 (no hemorrhage) and 1 (sparse hemorrhage).

Over the nine-month study period, 59 total cases were collected. Fifty-eight infants from birth to 11 months (median age two months) were included in the study. One case was excluded from the study because cause of retinal and NR/DRG hemorrhage could not be definitively attributed to trauma. Forty-eight infants died from natural, non-traumatic causes (non-trauma group) and ten from homicide or undetermined THI (trauma group). The number of NR/DRG recovered from each case varied. All 58 cases had multiple NR/DRG present in cervical and thoracic sections; 57 had lumbar NR/DRG, 56 had sacral NR/DRG, and 50 had cauda equina NR/DRG.

Every case in the trauma group displayed parenchymal NR/DRG hemorrhage, compared to 42% (20/48) of the non-trauma group. Hemorrhage in the trauma group was prominent (score=2) in 80% (8/10) of cases and sparse (score=1) in the other 20%. In those non-trauma group cases with hemorrhage, only 15% (3/20) showed prominent hemorrhage; scant hemorrhage was identified in the remaining 85% (17/20). Therefore, there is a significant association between increased severity of hemorrhage and presence of THI (p=.001).

These results support previous studies showing that prominent NR/DRG hemorrhage is a valid indicator of THI in infants. Moderate inter-rater agreement between two individuals at different levels of training highlights the utility of the proposed scoring methodology for general forensic practice; however, disagreement between no hemorrhage (score 0) and sparse hemorrhage (score 1) suggests the need for a stricter definition of sparse hemorrhage. Practically, only grade 2 hemorrhages, which had 100% agreement, appear to be a reliable indicator of significant pediatric head trauma. Further study is necessary to improve the scoring methodology and to more completely understand the connection between NR/DRG hemorrhage and fatal pediatric traumatic head injury.

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Traumatic Head Injury, Nerve Root, Ganglia Hemorrhage

H27 Accidental Injuries in Children: A Clinical Study for Improving the Forensic Interpetation of Child Physical Abuse

Federica Collini, MD, Via Mangiagalli 37, Milan 20133, ITALY; Enrico A. Muccino, MD, LABANOF, Via Luigi Mangiagalli, 37, Milan 20133, ITALY; Annalisa Cappella, BS, Via Mangiagalli 37, Milano 20133, ITALY; Lorenza Grappeja, MD, LABANOF, Via Luigi Mangiagalli, 37, Milan 20133, ITALY; Pasquale Poppa, BS, V. Mangiagalli, 37, Milan 20133, ITALY; Alessandra Kustermann, MD, SVSeD, Fondazione IRCCS Ca' Granda, Policlinico, Via della Commenda, 12, Milan 20122, ITALY; and Cristina Cattaneo, PhD, Universta Degli Studi Di Milano, Milan 20133, ITALY*

After attending this presentation, attendees will better understand how important it is to provide "control" data in order to differentiate between accidental and non-accidental injuries in the forensic evaluation of child abuse.

This presentation will impact the forensic science community by demonstrating which localizations are most commonly involved in all types of accidental trauma and their mode of production in children 3 years to 18 years of age, randomly selected from schools, with no history of abuse.

A recent study on this issue considered admissions to a medical center for both abuse cases and non-abuse "controls" according to similar standards; however, the existing literature has only briefly examined the pattern of injury distribution in accidental and non-accidental trauma in children generally under six years of age.[1-4] Moreover, the literature has especially focused only on bruising and its timing: this project seeks to extend the research to all types of skin injuries (recent, non-recent, and scars) and identify the most common manner of production and its frequency.[5,6] This could assist in the differential diagnosis between accidental and non-accidental trauma. Thus, the goal of this pilot control study was to highlight the anatomical location and the causes of accidental injuries based on medical history and the history of how specific trauma occurred in children less than 18 years of age.

Children were randomly recruited from three different comprehensive schools and nurseries, consisting of 205 individuals (103 males and 102 females). A physical examination, which took place after a consent form was signed by the parents, was performed on the entire body of the child, and all lesions and scars were photographed after the child/adolescent or parent had described how the lesion had occurred. The location of the injuries was recorded, according to nine anatomical areas (head/neck, anterior trunk, posterior trunk, upper limbs, lower limbs, hands, feet, pubis, and buttocks) and to 46 different sub-areas; the classification, dynamics (as told by the child or parent), frequency, and manner of production (sharp force injuries, blunt force wounds, and thermal injuries) were taken into consideration.

The study yielded a total of 1,381 lesions, with a mean of 6.3 injuries per person; however, 85% of the cases had a total number of less than 10 injuries. For 39% of the lesions, no information was given on the way in which the lesion had occurred, either because the child or parent did not remember or was not aware of the presence of the lesion or scar. Regarding the location of lesions, the results indicate that most of the lesions concern limbs (25% and 32%, respectively, for the upper and lower limbs), while the remaining injuries are divided as follow: 17% on the head/neck, 7% both on the anterior and posterior trunk, 10% on the hands, 2% on the feet, and 0.1% on the pubis and buttocks. If anterior/posterior location is considered, this study found only 36.3% of injuries in the posterior areas and 63.6% in the anterior areas of the body. This preponderance was true for all regions except for the upper limbs/hands, where 72% of wounds were on the posterior side of the body. The type of injury was divided as follows: 90% were blunt injuries (62.3% non-sport-related accidental injuries, 37.7% due to sports activities). Among the non-sport-related accidental injuries, 45% were excoriations, 36% lacerations, 15% bruises, 3% burns, and 1% fractures.

The results of this study show there is a peculiar anatomical distribution in accidental injuries, characterized by lesions to the upper posterior limbs and lower anterior limbs, as stated in literature but primarily only for blunt injuries. There was a high percentage of lesions for which there was no recollection: nearly 40% were of unknown origin. Blunt force trauma was the most representative (90%), while sharp and thermal injuries were significantly less represented. Moreover, among blunt force trauma, non-sport-related lesions were almost twice as high as the sport-related lesions.

The novelty of these data consists in the fact that all types of injury were considered (recent and old), along with their different modes of production (versus previous literature which considers only blunt force) and that a detailed account on how the trauma occurred was requested.

Finally, in child abuse cases, the history of how a lesion was produced is of great importance and the fact that for 39% of skin lesions there is no recollection by the child could be useful for interpreting abuse, since saying "I don't remember" does not necessarily mean "I don't want to tell."

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Child Abuse, Accidental Injury, Forensic Pathology

H28 A Unique Type of Birth Trauma Mistaken for Abuse

Carolyn V. Isaac, PhD, 1000 Oakland Drive, Kalamazoo, MI 49008-8074; Jered B. Cornelison, PhD, Western Michigan University School of Medicine, Dept of Pathology, 1000 Oakland Drive, Kalamazoo, MI 49008; and Joyce L. deJong, DO, WMU Homer Stryker MD, School of Medicine, Dept of Pathology, 1000 Oakland Drive, Kalamazoo, MI 49008*

After attending this presentation, attendees will understand a unique type of birth trauma caused by vacuum-assisted delivery that may be mistaken for child abuse.

This presentation will impact the forensic science community by increasing awareness of birth-related trauma and its presentation in the postmortem examination of infants.

Trauma during birth is a known risk with incidence of approximately 0.2% for unassisted vaginal deliveries and 1.1% for cesarean deliveries.[1,2] The risk of injury increases in instrument-assisted deliveries to 1.85%.[1] A consideration of birth trauma in potential cases of child abuse is an important differential diagnosis that must be explored. This is a unique case of a two-week-old infant that presented to the pediatric intensive care unit unresponsive, hypoglycemic, and hypothermic. Imaging revealed bilateral calvarial fractures underlying areas of soft tissue inflammation, raising concerns for a non-accidental etiology of the fractures that resulted in traumatic brain injury and death of the infant within a day of admission.

Upon presentation at the postmortem examination, the infant had diffuse dark purple skin discoloration with multiple areas of skin slippage. This degree of postmortem change was surprising considering the infant's death was pronounced approximately six hours prior. External examination revealed no other abrasions or other evidence of injury. Internal examination of the head confirmed the presence of the cranial vault fractures detected on imaging. These bilateral circular comminuted fractures of the parietal bones were observed in association with collections of blood beneath the scalp and generalized subarachnoid hemorrhage. Gross, microscopic, and histologic examination of the fractured bones was completed and revealed evidence of early osteogenic healing, suggesting an antemortem rather than a peri-mortem cause to the defects.

Further investigation revealed a circular defect approximately 22mm by 20mm of the right parietal and a somewhat figure eight-shaped defect on the left parietal measuring 40mm by 20mm. Gross and microscopic examination of these cranial defects showed outbending of bone along the perimeter, absence of ectocranial bone within the fracture border, and smoothed fracture margins with subperiosteal new bone formation indicative of healing. Histological samples demonstrated both osteoclastic and osteoblastic activity and confirmed the absence of ectocranial surface within the fracture margins. Based on these observations, the traumatic lesions occurred more than ten days prior to death.

A review of birth records revealed the infant was delivered via vacuum assist and cesarean section. Fractures associated with vacuum extraction are reported in the literature but are described as linear fractures, usually associated with a cephalohematoma. The external deformation of the fracture margins and circular morphology of the defects in this case are consistent with the type of cup that is utilized during vacuum extractions. Thus, the fractures present at autopsy were incidental findings of past birth trauma. Based upon bacterial cultures and neuropathology consultation, it was determined the infant died of gram-negative sepsis and meningitis due to *Enterobacter cloaecae* infection.

This unique case demonstrates the importance of considering birth trauma in a differential diagnosis in the determination of the cause and manner of death of an infant. While the type of cranial fracture reported here is rare, other injuries such as soft-tissue injury, clavicle fracture, nerve palsies, intracranial hemorrhage, humerus fracture, and linear or depressed skull fractures are considered common birth injuries and should be considered in potential cases of child abuse.

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Child Abuse, Birth Trauma, Fracture

H29 Histological Abnormalities of the Costochondral Growth Plate in Infants and Young Children

Sandacan Waduge, MD, King County Medical Examiner's Office, 325 Ninth Avenue, HMC Box 359792, Seattle, WA 98104; Micheline Lubin, MD, King County Medical Examiner's Office, 325 Ninth Avenue, HMC Box 359792, Seattle, WA 98104; and Richard C. Harruff, MD, PhD, King County MEO, 325 Ninth Avenue, Box 359792, Seattle, WA 98104*

After attending this presentation, attendees will better understand the normal microscopic anatomy of the growth plate and the prevalence of growth plate abnormalities in ribs of infants and young children examined by the King County Medical Examiner's Office (KCMEO) in Seattle, WA.

This presentation will impact the forensic science community by contributing to the understanding of growth plate abnormalities of ribs in infants and young children that may indicate subclinical vitamin D deficiency and vulnerability to skeletal injuries or susceptibility to natural disease.

Previous studies have correlated abnormalities in the growth plate of infant ribs with subclinical vitamin D deficiency and suggested an increased vulnerability to skeletal fractures and sudden infant death. The anatomy of the normal growth plate is best appreciated as a sequence of transition from cartilage to bone, in which the critical features are most readily evident in the Hypertrophic Zone (HZ), where chondrocytes are large and arranged in columns, and in the Osteogenic Zone (OZ), where osteoblasts deposit osteoid on spicules of calcified cartilage matrix.

This study is a retrospective review of autopsies of infants and young children less than three years of age to evaluate histological abnormalities of the growth plate and their correlation with demographic and biological parameters. The costochondral junction of the ninth rib was sectioned perpendicularly to its long axis, decalcified in 10% formalin and 10% formic acid, processed by standard histological methods, and stained with hematoxylin and eosin. The slides were evaluated according to seven previously described criteria, as follows: (1) loss of columnar arrangement of cells in the HZ; (2) increased density of cells in the HZ; (3) increased thickness of the HZ; (4) irregularity of the boundary of the HZ and the OZ; (5) tongue-like projections of cartilage extending from the HZ into the OZ; (6) presence of thin-walled vessels traversing from the HZ into the OZ; and, (7) excess osteoid in the OZ. Cell density and thickness of the HZ were measured quantitatively using digital images with calibrated micrometer scales.

Altogether, the study included costochondral segments from 160 decedents, 138 of whom were 12 months of age or younger, including deaths due to natural disease (19), injuries (10), undetermined causes including Sudden Unexpected Death in Infancy (SUID) and Sudden Infant Death Syndrome (SIDS) (105), and other causes including asphyxia (26). In these 160 cases, abnormalities of the growth plate according to the seven criteria were identified as follows: arrangement of cells in the HZ was disorganized in 40%; height of the HZ was increased in 14%; cell density in the HZ was increased in 9%; an irregular boundary between the HZ and the OZ was identified in 11%; invasion of blood vessels in the HZ was identified in 1.2%; tongue-like projections of cartilage in the OZ were identified in 1.2%; and excess osteoid in the OZ was identified in 1.2%. Of the 160 cases, 59% had no abnormality in any of the seven criteria, 20% had abnormalities in one criterion, 11% showed abnormalities in two, 8% in three criteria, and 1.2% in four criteria. Two cases had abnormalities in all seven criteria and thus fulfilled the histological features of rickets.

Growth plate abnormalities were identified only in infants 12 months or younger. No differences were identified in any age group according to race or gender. No differences in growth plate abnormalities were associated with weight, but marginally significant differences were associated with height. Only two cases had histological changes of rickets. Unexplained fractures were not found.

The results indicated that the ribs of children under one year of age are more likely to manifest subtle growth plate abnormalities compared to older children. This finding may be related to breast feeding in infancy and lack of vitamin D supplementation. Additional prospective studies are needed to correlate histological changes of the growth plate with nutrition, vitamin D levels, and vulnerability to skeletal fractures.

Pediatric Bone Development, Growth Plate Abnormality, Vitamin D Deficiency

H30 Risk Assessment for Asphyxia Without Doll Reenactments in Infant Deaths

Christopher Kiefer, MD, 1800 Shroyer Road, Apt 2, Dayton, OH 45419; and Kent E. Harshbarger, MD, JD, Montgomery Co Coroner's Office, 361 W 3rd Street, Dayton, OH 45402*

After attending this presentation, attendees will understand the importance of identifying the risk factors for positional asphyxia in infant deaths by examining the trends of Sudden and Unexpected Infant Death Investigation (SUIDI) in the last decade-and-a-half, over which period of time the number of Sudden Infant Death Syndrome (SIDS) cases has remained at historically low levels. This presentation will then provide the audience with an approach to SUIDI that produces desirable outcomes without the use of routine doll reenactments.

This presentation will impact the forensic science community by showing comparable outcomes in terms of identification of risk factors for asphyxial deaths in SUIDI cases between offices with and without a routine doll reenactment strategy and will lead attendees to consider the level of experience of the investigator before implementing a policy of routine doll reenactments; however, the conclusions in this study are not meant to diminish the importance of the doll reenactment for the purposes of creating a visual record of a SUIDI case, especially as a way of communicating the findings to the pathologist or other interested party. Finally, no discussion on the benefits of doll reenactments would be complete without addressing the emotional effects on both the investigator and caregiver.

The Montgomery County Coroner's Office (MCCO) in Dayton, OH, performed investigations in 52 sudden infant deaths from January of 2009 to July of 2015. The case records of sudden and unexpected infant deaths in this office were evaluated for the rate of identification of risk factors for positional asphyxia, which includes co-sleeping with an adult or sibling, sleeping on a couch or an adult bed, being found in the prone position or with the face against the mattress, cushion, pillow, or another person's body, and wedging. The approach taken to SUIDI by the MCCO does not include routine doll reenactments. Out of a total of 53 cases, risk factors of positional asphyxia were identified in 43 (81.1%), of which none involved the use of a doll reenactment, and of the 10 remaining cases, only one was subject to a doll reenactment. Previous studies have demonstrated that the risk factors for asphyxia can be identified in as many as 85% of cases of SUIDI when the strategy includes the use of routine doll reenactments.[1] In contrast, the MCCO relies on the death investigators' multi-year experience as police homicide detectives in order to conduct thorough investigations. The results of this study reveal comparable outcomes between the MCCO and the office of the previous study's authors, despite a disparity in the number of doll reenactments performed.

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SUIDI, Positional Asphyxia, Doll Reenactment

H31 Comparison of Safe vs. Unsafe Sleep Environments in Sudden, Unexpected Death in Infants

Michael D. Eckhardt, MD, Northshore Health Systems, 2650 Ridge Avenue, Evanston, IL 60201; Steven M. White, MD, PhD, County Cook OME, 2121 W Harrison Street, Unit D7, Chicago, IL 60612; Jon Gates, MD, Cook County Medical Examiner, 2121 W Harrison Street, Chicago, IL 60612; and Eric August Eason, MD, Cook County Medical Examiner, 2121 W Harrison Street, Chicago, IL 60612-3705*

After attending this presentation, attendees will understand the difference between safe and unsafe sleeping environments and their impact in the determination of cause and manner of death in infant deaths.

This presentation will impact the forensic science community by highlighting the role of an unsafe sleep environment in sudden, unexpected death in infants.

Distinguishing between natural disease and potential asphyxia in infant deaths can be problematic, if not impossible. Between 2011 and 2013, there were significant changes in the way infant deaths were investigated and how cause and manner of death were designated. The terms "Sudden Infant Death Syndrome (SIDS)," "Sudden, Unexplained Death in Infancy (SUDI)," "unknown natural causes," and "undetermined" have all been used somewhat interchangeably in cases in which there is no clear cause of death. These deaths were selected for study in more detail to see if there are any differences in sleeping position, demographics, and birth, medical, family, and social history between infants in "safe" versus "unsafe" sleeping environments that stand out.

A search of the Cook County Medical Examiner's Office database from 2011 to 2013 was performed for decedents between the ages of 0 and 12 months in which "SIDS," "SUDI," or "undetermined" were listed as the cause of death. The case files were reviewed and records made of demographic data, cause and manner of death, and data related to sleeping environment, circumstances surrounding death, birth history, medical history, family history, and social history of parents.

During the period of 2011-2013, there were 193 infants who died suddenly and unexpectedly in Cook County, Illinois. Of these cases, 44 were designated as accidental deaths with cause of death as asphyxia due to overlay, wedging, or suffocation. The remaining 149 cases included cases signed out as SIDS (n=16, 11%), SUDI (n=48, 32%), and undetermined (n=85, 57%). Of these 149 cases, there was an equal distribution of gender with 74 (50%) males and 75 (50%) females and the average age was 3.1 months (range 0.25 to 12 months). There were racial differences with 108 (72%) of the infants being black and 41 (28%) being white.

This study defined safe sleeping environments as a crib, bassinet, pack and play, baby swing, or car seat, while unsafe sleeping environments included bed-sharing with others, an adult-size bed, couch/sofa, play pen, in the arms of sleeping parents, and when the sleeping environment was unknown. Out of the 149 cases, there were 29 (19%) with safe sleeping environments and 120 (81%) with unsafe sleeping environments. There were no differences with regard to age, gender, race, or gestational age at delivery between the two groups. Interestingly, 43% of the infants in the safe sleeping group were exposed to cigarette smoking or illicit drugs before and after birth. Low birth weight was seen in 33% of the infants in the safe sleeping group compared with 20% in the unsafe group. With regard to how the infants were placed to sleep, 33% of infants in the safe group were placed to sleep prone, while 17% were placed prone in the unsafe group. Despite being in what is considered to be a safe sleeping environment, there was soft bedding present in approximately 99% of all cases. There were no differences between the two groups with regard to illnesses during infancy, prenatal care, and maternal education level.

The contribution of unsafe sleeping environments to infant mortality remains unclear. It is possible that in some cases, there were natural diseases or genetic abnormalities that contributed to death. Some benefit may be derived from genetic testing and a new policy includes saving tissue for this purpose.

Infant Death, Sleep Environment, SIDS/SUDI

H32 Unusual Blunt Force Trauma to the Cranial Vault: Investigation of an Atypical Infant Abuse/ Homicide

Jered B. Cornelison, PhD, Western Michigan University School of Medicine, Dept of Pathology, 1000 Oakland Drive, Kalamazoo, MI 49008; Carolyn V. Isaac, PhD, 1000 Oakland Drive, Kalamazoo, MI 49008-8074; Brandy Shattuck, MD, Western Michigan Homer Stryker MD, School of Med, 1000 Oakland Drive, Kalamazoo, MI 49008; and Joyce L. deJong, DO, WMU Homer Stryker MD, School of Medicine, Dept of Pathology, 1000 Oakland Drive, Kalamazoo, MI 49008*

After attending this presentation, attendees will better understand multiple unusual blunt force cranial injury patterns and manual asphyxiation, ultimately resulting in homicide. Attendees will also learn how a combination of microscopic and macroscopic examination contributed to interpreting an injury pattern that was sustained on multiple occurrences over an extended period of time.

This presentation will impact the forensic science community by highlighting a continued need for case studies to understand how unusual blunt force injuries can produce atypical fracture patterns. Interpretation is complicated within a context of repeated traumatic events.

A three-month-old infant was found unresponsive in a pack and play at home. The parents transported the infant to the hospital for evaluation. After extensive life-saving procedures, the infant failed to thrive and died. At postmortem examination, most body measurements fell well below expected growth rates (less than 5th percentile). The conjunctivae were pale with multiple petechiae and there were multiple petechiae on the skin of the face adjacent to the right eye. Areas of injury evident at autopsy included two areas of hemorrhage of the scalp, corresponding areas of hemorrhage of the soft tissues adherent to the skull, multiple skull fractures associated with the areas of hemorrhage, and attendant subarachnoid hemorrhage underlying the skull fractures. Multiple skull fractures and Classic Metaphyseal Lesions (CMLs) were also observed radiographically.

The vault portions of the parietals, frontal, occipital, and the left arm were removed at autopsy for gross, microscopic, and histological anthropological trauma analysis. The skeletal analysis revealed multiple healing fractures including seven fractures on the left parietal, six fractures on the right parietal, and one fracture on the occipital. Notably, six of the cranial fractures were linear fractures oriented perpendicular to the sagittal plane and extending to the sagittal suture. The pattern of these fractures are consistent with multiple compressive blunt force injuries to the head.

Healing CMLs were observed grossly and radiographically on the left proximal humerus, left distal radius, and left distal ulna metaphyses. The distal ends of the ulna and radius display rotational deviation and distortion with reactive bone along the shafts, suggesting previously healed fractures. The injuries indicate a rotational force applied to the infant's arm.

Microscopic and histological examination revealed that the fractures of the skull and left arm were in varying stages of healing. These different stages of healing are distinguished by: (1) an early stage in which the fracture margin is open with rounded fracture margins and subperiosteal bone along the fracture margins; (2) a middle stage in which the fracture margins exhibit intermittent bone bridging and subperiosteal reactive bone along the fracture margins; and, (3) a late stage in which the fracture margin is completely obliterated with persistent subperiosteal reactive bone. These results indicate the infant experienced multiple traumatic events during life but none of these directly contributed to death.

The diffuse petechiae of the face and conjunctivae indicate the infant died of asphyxia due to obstruction of airways. Law enforcement investigation produced reports of repeated manual compression of the head, neck, and chin of this infant by another individual. Such a mechanism of injury would account for the multiple cranial fractures and various stages of healing.

In summary, this individual demonstrates multiple fractures of the head and left arm in varying stages of healing, suggesting multiple traumatic events. More importantly, if the information from law enforcement is accurate regarding the mechanism of injury, forensic pathologists and anthropologists gain insight into the effects of low velocity and slow-loading compressive blunt force trauma on fracture patterning of an infant skull. Histological methods provide an opportunity for forensic anthropologists to understand the bone healing response to repeated injury of the cranial vault. Finally, this case study highlights the need for anthropologists and skeletal biologists to investigate cranial histological and gross healing responses and stages in infants and children.

Child Abuse, Blunt Force Trauma, Fracture Healing

H33 Infant and Child Deaths Associated With Drug Intoxication: A Series of Six Cases Over 15 Years in Eastern Virginia

Babatunde L. Stokes, MD, 830 Southampton Avenue, Ste 100, Norfolk, VA 23510; and Wendy M. Gunther, MD, OCME, Tidewater District, 830 Southampton Avenue, Ste 100, Norfolk, VA 23510-1046*

The goals of this presentation are to: (1) review pharmaceutical drugs, both prescription and over-the-counter, associated with infant and child deaths in medicolegal death investigations; (2) discuss manner-of-death determination in difficult cases associated with infant and child drug overdose deaths; and, (3) assess the role of non-lethal levels of drugs in infant and child deaths.

This presentation will impact the forensic science community by increasing awareness for clinicians and investigators of the presentation of infant and child deaths associated with pharmaceutical drugs, reviewing manner-of-death determinations, and discussing possible preventative measures.

Fatal drug overdose (prescription or over-the-counter drugs) is rare in infants and children. In the past 15 years, only six cases have occurred in the Tidewater district of Virginia's Office of the Chief Medical Examiner (OCME). The office serves a population of more than one million and routinely examines approximately 60 infants and children below 11 years of age each year, representing about 10% of all autopsy cases.

The ages of the decedents ranged in age from six weeks to eight years. Four cases involved prescription drugs and two involved over-the-counter drugs. No case was deemed a homicide. In three cases, diphenhydramine was intentionally administered by caretakers, but all the pharmaceutical drugs were either ingested accidentally, administered as part of medically supervised therapy, or the method of administration could not be determined. No case was associated with illegal drugs. An in-depth review of the following cases is offered for consideration.

A six-week-old male infant died in a warm room, positioned belly down, and covered with a blanket. He had fresh and healing rib fractures and dural hemosiderin without acute blunt force injury. Although homicide was suspected, the manner of death was eventually deemed undetermined due to the lack of acute fatal injury. Postmortem blood toxicology showed 0.1mg/L diphenhydramine with less than 5.0mg/L acetaminophen. Diphenhydramine should not be administered to neonates or infants. It likely played a role in death despite its low postmortem level, according to the pediatric forensic consultant.

A four-month-old child born with Prune Belly (Eagle-Barrett) Syndrome died from an accidental overdose of clonidine, due to mistakes made by the caretaker. The responsible parent transported her child by private vehicle to the hospital after he displayed lethargy. The child died after several days of hospital treatment with no admission blood available for analysis. Death was attributed to clonidine overdose based on history, medical records, and autopsy findings including centrilobular liver necrosis and infarcts of the liver, small bowel, and stomach, and hypoxic-ischemic brain histology. The manner was deemed accident.

A one-year-five-month-old boy was discovered unresponsive in his crib five hours after being laid down for a nap. Autopsy showed a respiratory tract infection not responsible for death. Postmortem toxicology showed an elevated but below the toxic range level of diphenhydramine and a fatal level of methadone. It was never determined how the child ingested the methadone. The manner remained undetermined after all investigation.

A two-year-old girl was found unresponsive the day after she was brought to the hospital with concern for possible ingestion of prescription medication. After a three-hour period of emergency room observation, she was sent home. The next day, after she was given acetaminophen and diphenhydramine for a fever, she was found unresponsive. Autopsy identified back and upper arm scars of concern for possible child abuse and a fatal overdose of the components of a buprenorphine-naloxone combination prescribed a family member for opiate maintenance. The family felt the fatal overdose must have occurred prior to the emergency room visit, which was deemed not possible by the forensic pathologist. The manner was undetermined.

A six-year-old boy with a complex psychological and medical history had been prescribed quetiapine and clonidine. He was sleepy the day before death. Investigation suggested quetiapine overdose based on toxicology, pill counts with missing tablets, history, and negative autopsy. The manner was undetermined.

An eight-year-old girl underwent a dental procedure and stopped breathing during treatment. An initial lethal level of chloral hydrate was not confirmed on re-testing; hydroxyzine in blood, bile, and liver presented a confusing picture. Death was thought in the end to be due to complications of dental sedation using chloral hydrate, hydroxyzine, and nitrous oxide. The manner was deemed accident.

Fatal drug overdose in infants and young children are not common. Prevention of accidental ingestion should be geared toward educating parents and caregivers of proper drug-dosing instructions, educating parents and caregivers of when certain medication can and cannot be given to infants and young children (e.g., diphenhydramine should not be given to infants), and placing all medications out of reach of young children.

Clonidine, Pediatric Overdose Deaths, Infant Drug Deaths

H34 Undiagnosed Metabolic Cardiomyopathy as a Cause of Pediatric Sudden, Unexpected Death: Case Report and Review of the Literature

Lauren M. Woertz, 2121 W Harrison Street, Chicago, IL 60612; Steven M. White, MD, PhD, County Cook OME, 2121 W Harrison Street, Unit D7, Chicago, IL 60612; and Audrey Deeken-Draisey, MD, Northwestern Memorial Hospital, 208 W Washington Street, Unit 1206, Chicago, IL 60606*

After attending this presentation, attendees will understand the potential for undiagnosed cardiomyopathy resulting from Inborn Errors of Metabolism (IEM) as a cause of sudden, unexpected death in children and will recognize the gross and histologic findings suggestive of cardiomyopathy resulting from a metabolic disorder.

This presentation will impact the forensic science community by enhancing the recognition of pediatric cardiomyopathies occurring due to metabolic disorders as a cause of sudden cardiac death in children. The forensic community will understand the diagnostic methodologies required for the postmortem detection of metabolic disorders. This presentation also highlights the fact that potentially lethal IEM may not be detected by routine neonatal screening.

Pediatric cardiomyopathies are a relatively rare and varied group of disorders that differ widely in their causes and outcomes. They are also an important cause of morbidity and mortality in this population, and children with cardiomyopathy are at risk for sudden cardiac death.[1] While the etiology for the development of cardiomyopathy is varied, IEM have been shown to cause a substantial proportion of pediatric cardiomyopathies.[2] The detection methods for identification of IEM have improved, although there remains a significant risk for complications if the cardiomyopathies remain undiagnosed and untreated.

Inborn errors of metabolism in pediatric patients have recently been shown to account for 26% of hypertrophic cardiomyopathies and 16% of dilated cardiomyopathies.[3] IEM occur in approximately 1 in 4,000 newborns and comprises over 1,000 unique diagnoses. Forty types of IEM have been documented to cause cardiomyopathy, including fatty acid oxidation defects, organic acidemias, glycogen storages diseases, amino acidopathies, and peroxisomal, mitochondrial, and lysosomal storages disorders. Only about 5% of IEM cases are associated with cardiomyopathies and rarely is the heart the only affected organ.[4] Most pediatric cardiomyopathies are diagnosed early in life due to the development of symptoms; however, they may go undiagnosed, first presenting at autopsy. In addition to determining the cause of death, establishing a diagnosis of cardiomyopathy due to IEM may have important implications for surviving family members and may influence family planning in cases of hereditary IEM.

This presentation pertains to the case of a 21-month-old Caucasian female with no significant past medical history. The child experienced two days of flu-like symptoms including a cough, congestion, and fever prior to developing labored breathing and becoming unresponsive in the arms of a caregiver. She died a mere 22 minutes later in the hospital. There was no family history of heart disease or sudden infant death. The child had normal development, met appropriate milestones, and the newborn laboratory screen performed at birth was normal. Postmortem toxicology and microbiology tests were non-contributory. At autopsy, the heart was dilated with a globoid shape, and the formalin-fixed specimen weighed 123 grams (expected 33-89 grams). Histologic examination of the heart revealed vacuolated cardiac myocytes in a patchy distribution. An Oil Red O stain performed on a frozen section of formalin-fixed tissue revealed intracellular lipid accumulation within the vacuoles; Periodic Acid-Schiff (PAS) stains with/without diastase were negative. Histologic examination of the liver revealed steatosis in a centrivenular pattern. The gross and histologic findings of the heart were consistent with dilated cardiomyopathy due to a probable metabolic disorder of unknown etiology. Intracellular lipid accumulation can be seen in both lipid-storage disorders and mitochondrial disorders. Whole genome sequencing is currently pending to further classify the specific metabolic disorder that contributed to the decedent's death.

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Metabolic Disorder, Cardiomyopathy, Pediatric Sudden Death

H35 Use of an Automated, Nested, Multiplex, Respiratory Pathogen Polymerase Chain Reaction (PCR) Panel Postmortem in the Pediatric Forensic Setting

Tiffany Baker, 165 Ashley Avenue, Ste 309, Charleston, SC 29425; Cynthia A. Schandl, MD, PhD, Medical University of SC, 171 Ashley Avenue, Ste 309, MSC-908, Charleston, SC 29425; S. Erin Presnell, MD, MUSC Department of Pathology, Autopsy Section, 171 Ashley Avenue/MSC 908, Charleston, SC 29425; James Madory, DO, Medical University of South Carolina, 171 Ashley Avenue, Ste 309, MSC 908, Charleston, SC 29425; and Nicholas I. Batalis, MD, Medical Univ of South Carolina, 171 Ashley Avenue, Ste 309, MSC 908, Charleston, SC 29425*

After attending this presentation, attendees will understand the benefits of using an automated, nested, multiplex, respiratory pathogen PCR panel in determining cause of death in decedents, ages 0 to 12 years.

This presentation will impact the forensic science community by demonstrating the utility of an automated, nested, multiplex, respiratory pathogen PCR panel in determining cause of death in a pediatric population.

Molecular diagnostic techniques have only recently become useful as rapid diagnostic tools due to innovations in automation, decreasing cost, and resultant increased accessibility. Several studies have been published regarding the use of Polymerase Chain Reaction (PCR) to identify respiratory pathogens postmortem.[1-11] No prior study has been conducted using an automated, nested, multiplex, respiratory pathogen PCR panel on fresh postmortem samples. The potential benefits of such analysis include a greater breadth of pathogen detection. As costs have decreased, implementation of sophisticated molecular testing in the postmortem setting has become possible. The purpose of the current study was to determine the utility of automated, nested, multiplex respiratory pathogen PCR panels in in determining cause of death.

In order to ascertain the utility of this technology in determining cause of death, such tests were performed on coronial decedents, ages 0-12 years, from January 1, 2009 to June 6, 2015, in the Medical and Forensic Autopsy Section at the Medical University of South Carolina. Cases were selected for PCR on the basis of pathologist suspicion of potential respiratory illness at time of death. Samples were acquired via postmortem mucosal swabs from nasopharyngeal, tracheal, or bronchial regions. Samples were analyzed using an automated, nested, multiplex PCR panel of respiratory pathogens.[12] Over the course of the study, the respiratory pathogen panel detected from 12 to 17 potentially pathogenic agents, with the most recent panel including Adenovirus, *Bordetella pertussis*, *Chlamydia pneumoniae*, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Influenza A and B, *Mycoplasma pneumoniae*, Metapneumovirus, Parainfluenza 1-4, Respiratory Syncytial Virus, and Rhinovirus/Enterovirus. The contribution of each positive PCR result to the cause of death was critically examined and interpreted based on the autopsy findings and known circumstances surrounding death.

A total of 37 cases warranted a respiratory pathogen PCR panel in 0-to-12-year-old decedents. Of those, 17 (45.9%) yielded positive PCR results. In 41% (7/17) of these cases, the cause of death was associated with the respiratory illness detected by the PCR panel. Five (29.4%) of the cases with a positive PCR result were determined not to play a significant role in cause of death. The remaining five PCR positive cases remained undetermined regarding the role of the detected pathogen in the death. Potentially pathogenic agents detected included Rhinovirus/Enterovirus (12 cases), RSV (4 cases), Adenovirus (2 cases), Coronavirus NL63 (2 cases), and influenza B (1 case). Co-infections were documented in three cases. Of the 26 cases in which the decedent was under one year of age, 11 (42.3%) had a positive viral PCR result. Thirteen decedents less than one-year-old were assigned sudden unexplained infant death or "undetermined" as cause of death; 5 of those 13 (38.5%) cases had a positive viral PCR result.

Results indicate that an automated, nested, multiplex, respiratory pathogen PCR panel currently used for diagnostic purposes in living patients can be applied at time of autopsy to aid in determining cause of death. Furthermore, regular use of such PCR panels postmortem could have a significant impact on our knowledge of public health and epidemiology.

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Automated PCR, Respiratory Pathogen, Pediatric Cause of Death

H36 A Retrospective Study of Natural Causes of Sudden Unexpected Infant Deaths in Hubei, China

Xiang Zhang, MD, OCME, 900 W Baltimore Street, Baltimore, MD 21223; Ling Li, MD*, OCME, State of Maryland, 900 W Baltimore Street, Baltimore, MD 21223; Guoqiang Qian, MD*, Public Security Bureau of Jinhua City, 1055 Bayi Bei Road, Wucheng District, Jinhua 321000, CHINA; Zhiyong Yang, MD*, Public Security Bureau of Tianjin, 440 Anshan Western Road, Nankai District, Tianjin 300193; Tiantong Yang*, Haidian District, 26 Houtun South Road, Beijing 100192, CHINA; and Zhaoming Guo, MD*, Institute of Evidence Law and Forensic Science, NO.26, Houtun South Road, Haidian District, Beijing 100192, CHINA*

After attending this presentation, attendees will better understand the epidemiological characteristics and pathological findings of sudden unexpected infant death caused by natural diseases in China.

This presentation will impact the forensic science community by providing attendees with a better understanding of why Sudden Infant Death Syndrome (SIDS) is exceptionally rare among Chinese infants when compared to Western infants. This presentation will also demonstrate the reasons for the rarity of SIDS in China.

The importance of forensic investigation and autopsy in cases of sudden unexpected infant death has received public attention only in the past 10 years in China.[1] Risk factors for sudden infant death have been investigated by many countries.[2,3] This study is presented to describe the epidemiological characteristics and pathological findings of sudden infant death cases investigated by the Department of Forensic Medicine at the Tongji Medical College in Hubei, China.

A retrospective search of sudden infant deaths caused by natural diseases from forensic autopsy cases was performed at the Department of Forensic Medicine, Tongji Medical College in Hubei province, China. Over a seven-year period, a total of 55 infants who died suddenly and unexpectedly were determined to be natural deaths after a thorough investigation and autopsy examination. The ages ranged from newborn to 12 months. Of the 55 cases, the majority of deaths (65%) (36/55) occurred in the neonatal period, 18% (10/55) were in the first six months of life, and the remaining cases 17% (9/55)were between seven months and one year of age. There were 43 males and 12 females (M:F=3.6:1). The most common natural causes of sudden neonatal death were lobar pneumonia due to amniotic fluid aspiration (N=13) followed by congenital abnormalities (N=9), prematurity (N=8), interstitial pneumonia (N=3), bronchopneumonia (N=1), exsanguinations due to umbilical cord bleeding (N=1), and tetanus (N=1). The two leading causes of sudden infant death, age 1 month to 12 months were interstitial pneumonia (N=10) and meningitis (N=2). There was only one SIDS death.

Compared to Western countries, infectious diseases are the most common cause of death in infants in China. SIDS was believed to be exceptionally rare among Chinese infants. There was only one infant whose death was attributed to SIDS during the past seven years. The reasons for the rarity of SIDS death in China include: (1) a very low infant autopsy rate; (2) under-diagnosis (most of the physicians, including forensic pathologists, do not believe in a SIDS diagnosis); (3) over-diagnosis of interstitial pneumonia as a cause of sudden infant death; and, (4) better sleeping arrangements, such as supine sleeping on firm beds with close supervision by the parents.

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Forensic Science, Sudden Infant Death Syndrome, Forensic Autopsy

H37 Female Suicides in Southern Marmara: A Retrospective Analysis of 8,048 Cases Between 2009 and 2014

Nursel Turkmen Inanir, Uludag University School of Medicine, Dept of Forensic Medicine, Bursa, TURKEY; Murat S. Gürses, MD, Uludag University School of Medicine, Dept of Forensic Medicine, Bursa, TURKEY; Selcuk Cetin, MD, Gaziosmanpasa University Medical School, Dept of Forensic Medicine, Tokat, TURKEY; Mustafa N. Ural, Uludag University School of Medicine, Dept of Forensic Medicine. 16059, Görükle, Bursa 16059, TURKEY; Eser Bayraktar, MD*, Uludag University School of Medicine, Dept of Forensic Medicine, Bursa, TURKEY; Bulent Eren, Council of Forensic Medicine of Turkey, Bursa Morgue Dept, Bursa, TURKEY; and Recep Fedakar, Uludag University School of Medicine, Bursa, TURKEY*

After attending this presentation, attendees will understand the importance of the prevention of female deaths due to suicides.

This presentation will impact the forensic science community by providing results from a six-year retrospective study in the vicinity of Bursa, Turkey.

The high rates of suicide and attempted suicide which characterize this topic is a significant public health problem.[1] Hanging is the most common method of suicide in Turkey and worldwide.2 Researchers investigated gender-specific association between risk factors and suicide, controlling for demographic factors. In this regard, suicide deaths for females were associated with mental health service use in both younger and older age groups.[3] Additionally, female suicides have higher rates of depression than males in the literature.[4] Menon et al. reported that certain differences were seen to be present between male and female suicide attempters with regard to baseline personality attributes.[5]

In the present study, 8,048 forensic autopsy cases performed in the Bursa morgue Department of the Council of Forensic Medicine, Turkey, from 2009 to 2014 were evaluated retrospectively for female suicidal death cases. Two hundred fifty-five suicide cases were analyzed for age, cause of death, time of year/month, hospitalization period, autopsy findings, putrefaction, toxicology results, medical history, marital status, cohabitation, death scenes, and detection of sperm on vaginal/anal swabs. The age classification was determined according to youth, young adult, middle adult, older adult to average retirement age, and retired (1-14, 15-24, 25-44, 45-64, and 65 years and older). Statistical Package for the Social Sciences (SPSS) statistics 20.0 was used for statistical analysis. The mean age of the cases was 42.08±19.66 years and ranged between 12 years and 87 years. The most common suicidal attempts encountered were hanging (69.4%), followed by drug intoxication (7.8%), firearms injury (7.1%), pesticide poisoning (5.9), and falls from height (5.5%). Only one case had self-inflicted stab wounds. Of the studied cases, 31.4% were under medical treatment and the most common cause of disease was psychiatric disorders. The most common age group for hanging was young adulthood and, when compared to other age groups, this data was statistically significant.

The hanging ratio shows that significant differences between suicide attempts and personality attributes such as age and psychiatric treatment were identified as important for female suicides. This research seeks to evaluate personality factors, differences in female suicides between suicide attempts, personality attributes, and the association of suicide risk.

Suicide-related deaths, especially female suicides, are still an important public health issue in society. All safety regulation measures must be taken by all related authorities and more comprehensive current studies must be undertaken for causes of female suicide.

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Autopsy Results, Suicide, Hanging

H38 Early Ischemic Heart Injury: An Immunohistochemical Study of a Paradigmatic Case

Silvia D. Visonà, MD, Via Guicciardini 9 Bis, Varese, ITALY; David Forni, MD, University of Pisa, via Roma, 55, Pisa 56100, ITALY; Giovanni Pierucci, MD, University Of Pavia, Via Forlanini, Pavia 27100, ITALY; Luisa Andrello, MD, Magenta Street 25/I, Olgiate Olona, Varese 21057, ITALY; and Antonio M.M. Osculati, MD, Via Forlanini 12, Pavia 27100, ITALY*

The goal of this presentation is to provide the results of an investigation concerning immunohistochemical methods that may allow for an accurate postmortem diagnosis of early ischemic lesions of the heart muscle.

This presentation will impact the forensic science community by providing the results of a postmortem immunohistochemical study of the myocardium in a case of sudden cardiac ischemic death, compared to a control matched by age and sex, resulting from a fall from a height.

Introduction: Ischemic heart disease is the major cause of sudden cardiac death. In forensic pathology, The need for new methods is particularly crucial in cases in which ischemic lesion occurs shortly before death: often, in very early ischemia (less than three hours), the damage can't be detected by light microscopy. On the basis of a previous immunohistochemical study, this study attempted to discover if a number of immunohistochemical markers could be useful in order to differentiate ischemic areas from well-perfused ones.

The goal of this presentation is to provide the results of a postmortem study of the myocardium in a case of sudden cardiac ischemic death compared to a matched control who died from a fall from a height.

Materials and Methods: A forensic autopsy was performed 72 hours after death. The whole heart was fixed and specimens for microscopic examinations were collected from each part of the heart. Each section was stained with Hematoxylin-Eosin (H&E) and with the following primary antibodies: antifibronectin, anti C5b9, anti Cx43, antiNpCx43, and antiZO1. Immunopositives of each marker in the myocardium were semi-quantitatively graded.

Results: A 39-year-old woman went to the hospital complaining of chest pain. The objective examination, blood exams, and Electrocardiogram (ECG) didn't point out any pathological findings. A few hours later, she was discharged. The next day cardiologists performed an ECG and an echocardiography (referenced as negative); the pain was diagnosed as angina pectoris and a stress test was scheduled. The morning after, the woman suddenly died at home. At the autopsy, the heart showed an increase of the left ventricle wall thickness and the subtotal stenosis of the interventricular anterior branch of the left coronary. At H&E staining, the myocardium showed intimal thickening of the walls of small arteries, contraction bands and fibers disarrangement, without necrosis. Immunostaining: Fibronectin (FN) — neither the scoring nor the distribution pattern seem to show any relationship with the site of ischemia. Among the case samples, different patterns of distribution could be noticed, but not related to the ipoperfused area. C5b9 was negative in the cytoplasm, in both the case and the control specimens. CX43, npCx43, ZO1 — in the specimens from the diseased area, a less marked and disarranged Cx43 staining was observed, whereas NpCX43 in these specimens showed a more marked positivity and disomogeneous distribution. ZO1 showed a ill-scattered pattern in the specimens collected from the ischemic area; it was weak in the control specimens.

Discussion: On the basis of a previous immunohistochemical study, attempts were made to ascertain if these markers could be useful in order to differentiate ischemic areas from the well-perfused areas.[1] By the analysis of many specimens from one heart (obtained from a patient whose clinical data, diseased coronary artery, and ipoxic-ischemic area were known), the study is free of any influence by the postmortem interval or by the time of fixation. The most useful marker seems to be the combination of Cx43 and its de-posphorilated form (npCx43). In the normal heart, Cx43 is phosphorylated and localized at the Intercalated Discs (ID); stimuli such as ischemia and hypoxia induce non-phosphorylation and redistribution to the cytoplasm and/or lateral cell border of cardiomyocytes.[2-4] In animal models, decreased Cx43 and increased npCx43 at ID were detected 15min after the beginning of ischemia.2 The less-defined pattern of npCx43 might indicate its redistribution from the cell junction to the cytoplasm. As the ZO1 binds and regulates the Cx43, it can be another marker of this junction disruption. In this case, a clear correlation between ZO1 pattern and ischemia was not observed.[5] All of these markers, despite the interesting findings about their distribution, have the disadvantage of being in any case positive and the scoring could be influenced by many factors, such as technical or operator-related issues.

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Early Ischemia, Myocardial Ischemia, Immunohistochemistry

H39 Saddle Pulmonary Embolism With Paradoxical Coronary Artery Embolism Through a Patent Foramen Ovale: A Case Study

Amber L. Achesinski, BS, Eastern Virginia Medical School, 604 Fairfax Avenue, Norfolk, VA 23507; Catherine B. Pearman, MPAS, Eastern Virgina Medical School, School of Health P, 651 Colley Avenue, Norfolk, VA 23507; and Wendy M. Gunther, MD, OCME, Tidewater District, 830 Southampton Avenue, Ste 100, Norfolk, VA 23510-1046*

The goals of this presentation are for attendees to: (1) consider the possibility of coronary artery embolism through a patent foramen ovale in sudden death from thromboembolism; (2) recognize the autopsy appearance of a thromboembolus propagating through a foramen ovale; and, (3) identify risk factors for deep venous thrombosis that could lead to life-threatening pulmonary embolism.

This presentation will impact the forensic science community by assisting attendees to: (1) recognize coronary artery embolism as a potential complication of patent foramen ovale in thromboembolism cases; (2) identify its role as a contributor to sudden death; (3) consider underlying natural causes of sudden death in patients presenting in motor vehicle accidents or by other accidental mechanisms; and, (4) detect unusual complications of pulmonary thromboembolism.

A 35-year-old White male with a medical history of knee surgery and a vague history provided by the family of an episode of severe meningitis a number of years ago was found in cardiac arrest on the side of the road after his car slowed below highway speeds and ran up onto the curb. There was reportedly minor damage to the vehicle and no major injuries noted on the patient. Cardiopulmonary resuscitation by Emergency Medical Services (EMS) and the local emergency room staff was not successful. The day prior to the fatal incident, the decedent had complained to his wife of a swollen right leg, shortness of breath, and chest pain. His wife strongly encouraged him to seek medical care. On the day of the incident, she reported that he was on his way to pick up diapers and then drive himself to the emergency room.

Upon autopsy, a saddle pulmonary embolism was discovered. As was suspected based upon the health complaints prior to death, a completely occlusive thrombus occupied the right pulmonary artery with distention and dilatation, as well as an incompletely occlusive thrombus in the left pulmonary artery. Histology confirmed the left thrombus was close to the hilum, with evidence of scar tissue and recent thrombus in the artery, suggesting a prior partial thromboembolism at that site.

Beyond the saddle embolism, findings were atypical. A patent foramen ovale was noted with a large paradoxical embolus extending from right atrium to left atrium and a thrombus noted in the circumflex coronary artery. Also of note, not only the right but both lower extremities showed Deep Venous Thrombosis (DVT); it was not possible to determine which leg supplied the embolus which was ultimately responsible for his death. Histology of the deep veins in the legs showed incompletely occluded veins with a clot appearing similar to the paradoxical embolism and no endothelialization (suggesting no old clot) and no evidence of previous endothelial injury.

Saddle embolism with paradoxical embolism in the coronary artery is a rare complication of DVT and patent foramen ovale. While a patent foramen ovale can be present in up to 30% of the population under 30, it is suggested that "paradoxical coronary embolism accounts for 10%-15% of all paradoxical emboli," but is more common in patients under the age of 35.[1,2] While patent foramen ovale and pulmonary embolism are both common occurrences, the combination of saddle pulmonary embolism leading to death in a patient with patent foramen ovale and coronary artery embolism is an anomaly with few documented cases.

Virchow's triad, the set of risk factors that can help explain the formation of a thrombus, includes venous stasis, injury to the endothelium, and a tendency for hypercoagulability, such as malignancy or clotting factor deficiency.[3] In review of the patient's risk factors for DVT, a history of knee surgery was obtained; however, since no surgical scars could be identified on external examination, the decedent's knee surgery may have been minor. He also had a distant history of severe meningitis and it is possible that during that time he developed an undetected and untreated DVT and/or pulmonary embolism while he was septic and immobilized. This may have put him at risk for further thrombus, supported by the histology report suggesting new thrombus at the site of prior thromboembolism in the lung. It is also possible that the decedent had an unknown clotting factor deficiency which put him at risk for thrombosis. Relevant family history includes the patient's father's development of a partial stroke, in the absence of hypertension and hypercholesterolemia, which resolved with treatment a few months prior to the decedent's fatal event. Liver samples were retained at -70°F for genetic testing availability.

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Pulmonary Embolism, Paradoxical Embolism, Patent Foramen Ovale

H40 Massive Fetomaternal Transfusion (FMT): Case Reports and Review of the Literature

Silvana Temi, MD, University of Torino - Sezione di Medicina Legale, c.so G. Galilei, n. 22, Torino 10121, ITALY; Giovanni Botta, MD, Department Of Pathology, Oirm Sant'anna, C.so Spezia, #60, Torino 10126, ITALY; and Giancarlo Di Vella, MD, PhD*, University of Torino, Dept Public Health Sciences, Sezione di Medicina Legale, Corso Galileo Galilei 22, Torino 10126, ITALY*

After attending this presentation, attendees will develop a better understanding of the importance of an accurate protocol to identify cases of FMT.

This presentation will impact the forensic science community by emphasizing the importance of requiring the Kleihauer-Betke Test (KBT) to confirm cases of FMT.

Introduction: FMT is the transplacental passage of fetal blood cells to the maternal circulation due to disruption of fetal vessels within the chorionic villi. The passage of fetal blood into maternal circulation is often asymptomatic due to the low volume of blood transfused. In some cases, it would seem that intervillous thromboses, present in these asymptomatic cases of FMT, probably serves as a protective mechanism to limit a possible massive hemorrhage. The incidence range is 3/1,000 for 30ml of fetal blood loss and 1/1,000 if the loss exceeds 80ml. The latter case usually leads to fetal death. FMT is associated with events or conditions that lead to stillbirth or neonatal death such as placental disorders, abdominal trauma, or alloimmunization. It can be suspected in cases of neonatal anemia, decreased or absent fetal movements, or hydrops fetalis. The diagnosis is difficult because of the limits imposed by available tests. The test used most often is the quantitative KBT. This test relies on a cytochemical process that allows differentiation of the adult red blood cells from fetal erythrocytes on a maternal blood smear. Although valid, this test has many disadvantages; it is time intensive and overestimates maternal conditions leading to HbF production.

Goals: Etiology, clinical presentation, obstetrical antecedents, and outcomes of pregnancies complicated by large FMT were reviewed in the Hospital Sant'Anna in Turin, Italy, and compared to literature.

Materials and Methods: A descriptive study was conducted by analyzing autopsies performed at the Gynecological and Obstetrical Hospital Sant'Anna in Turin from 2004 to 2014. Cases of FMT were extrapolated and attention was focused on cases of perinatal death occurring between the third trimester of pregnancy and the first week of extra-uterine life.

Results: From 2004 to 2014, 3,395 autopsies were performed and among these were 456 perinatal deaths. There were 12 cases of suspected FMT with an incidence of 2.6/100 perinatal deaths. The age range of the women was between 22 years and 40 years, with an average age of 32 years. Their countries of birth were Italy (8), Romania (2), Morocco (1), and Tunisia (1). Three women were primiparas; five were experiencing their second pregnancy; two, their third pregnancy; and two previously had an abortion. Ten cases out of twelve were stillbirths; the other two were neonatal deaths. Six women were Rhesus -, the other six were Rhesus +. Indirect Coombs tests were positive in three cases and negative in the remainder. Amniocentesis was performed in only two of the pregnancies. Decreased or absent fetal movements had been perceived in each of these pregnancies. Two women had beta thalassemia. Histological examination of the placenta presented some common traits. Intervillous hemorrhages and villous immaturity were evident in all cases. Erythroblastosis and corangiosis were noted in some cases. The results of the KBT were positive in eight cases, negative in three cases, and the test had not been performed in one case.

Conclusions: The variability of the predisposing factors and the characteristics of women contributed to a difficulty in diagnosing FMT. In cases where there are characteristics of a pale fetus with pale viscera, FMT must be suspected. A positive KBT result is essential for confirming diagnostic suspicions, especially in cases with poor blood transfusion. FMT is a rare, dramatic, and underestimated event that often leads to death of the fetus. It is important to use KBT to confirm the diagnosis of every case of suspected FMT. It is recommended that the KBT test be included in all stillbirth protocols.

Fetomaternal Transfusion, Kleihauer-Betke Test, Perinatal Deaths

H41 The Prevalence of Paraphernalia Found at the Scene of Drug-Related Deaths

Jason Gene Lozano, MD, 9002 Indigo Lake, San Antonio, TX 78229; Kimberley Molina, MD, BCMEO, 7337 Louis Pasteur Drive, San Antonio, TX 78229; and Nicole L. Healy, BS, Bexar County Medical Examiner's Office, 7337 Louis Pasteur Drive, San Antonio, TX 78229*

After attending this presentation, attendees will better understand the prevalence of drug paraphernalia found at the scene of drug-related deaths and at the scene of deaths caused specifically by heroin, cocaine, or heroin and cocaine toxicity or abuse.

This presentation will impact the forensic science community by providing objective data to support long-held beliefs that drug-related deaths and deaths caused specifically by heroin, cocaine, or heroin and cocaine toxicity or abuse are characterized by the presence of drug paraphernalia at the scene.

Despite the recent attention given to designer drugs, heroin and cocaine continue to be popular among recreational drug users and many drug-related deaths are attributable to heroin and/or cocaine use. The use of heroin and/or cocaine often involves paraphernalia such as needles and syringes, pipes, spoons, et cetera. Recent recreational drug use is strongly suspected when a body is found with nearby drug paraphernalia. The presence of drug paraphernalia may assist and direct toxicological examination and deaths may be classified as drug-related even if the drug is not present in the toxicological examination.

In this study, deaths reported to the Bexar County Medical Examiner's Office (BCMEO) between August 2010 and December 2014 that were drug-related and in which a scene investigation occurred were identified. A total of 815 cases were identified of which 814 indicated the presence or absence of drug paraphernalia. Of the drug-related cases, 194 (24%) had drug paraphernalia found at the scene. The 815 drug-related cases were further scrutinized and limited to cases where the direct cause of death was heroin, cocaine, or heroin and cocaine intoxication or abuse. These cases accounted for 325 deaths, of which 122 (38%) had drug paraphernalia found at the scene. Heroin intoxication accounted for 162 deaths, of which 75 (46%) had drug paraphernalia found at the scene. Heroin intoxication or abuse accounted for 162 deaths, of which 75 (46%) had drug paraphernalia found at the scene. Cocaine intoxication or abuse accounted for 97 deaths, of which 21 (22%) had drug paraphernalia found at the scene. Heroin and cocaine intoxication or abuse accounted for 66 deaths, of which 26 (39%) had drug paraphernalia found at the scene.

In conclusion, this study provides objective data regarding the prevalence of drug paraphernalia found at the scene of drug-related deaths. As in previous studies, drug paraphernalia is found in only a minority of drug-related deaths (24%). Death due directly to cocaine intoxication showed a similar trend with drug paraphernalia found in 22% of these cases. Deaths due directly to heroin toxicity had paraphernalia present in almost half of scenes investigated (46%).

Cocaine, Heroin, Paraphernalia

H42 Sudden Cardiac Death (SCD) Visualized by Postmortem Magnetic Resonance Imaging (PMMRI) — How to Make the Invisible Visible

Christian Jackowski, MD, EMBA, Institute of Forensic Medicine, University of Bern, Bühlstr. 20, Bern, Canton Bern, SWITZERLAND; Nicole Schwendener, HF, Institute of Forensic Medicine, University of Bern, Bühlstr. 20, Bern 3012, SWITZERLAND; Anders Persson, MD, PhD, CMIV, Linköpings Universitet/US, Linköping 581 85, SWEDEN; and Wolf-Dieter Zech, MD, University of Bern, Institute of Forensic Medicine, Dept of Forensic Medicine and Imaging, Bühlstrasse 20, Bern 3012, SWITZERLAND*

After attending this presentation, attendees will better understand the possibilities of PMMRI for the detection and visualization of very early stages of myocardial infarction as a possible source for lethal ventricular arrhythmias often appearing as SCDs.

This presentation will impact the forensic science community by introducing a solution for a long-term problem, namely the impossibility to morphologically visualize an SCD postmortem.

SCD cases have challenged forensic pathologists for several decades as they often do not present with any myocardial finding at autopsy and/or histology. Lethal arrhythmic events are thought to cause these sudden deaths. Since PMMRI was implemented on a routine basis in forensic institutes, it was recognized that postmortem MR exams not only show chronic, subacute, and acute infarction but often also show myocardial findings in SCD cases which may represent very early (peracute) stages of myocardial infarctions thus far invisible at autopsy and/or histology.1-7

Therefore, this study investigated whether there is a correlation between these PMMRI findings (invisible at autopsy) and coronary artery findings that may be able to explain an early myocardial ischemic event as a possible source for a lethal arrhythmic event.

In 136 human forensic corpses, a postmortem cardiac MR examination was performed prior to forensic autopsy. Short-axis and horizontal long-axis MR images were acquired *in situ*. PMMRI findings were correlated to the autopsy findings.

In 76 study cases, myocardial findings could be documented and correlated to the autopsy findings. Within these 76 study cases, a total of 124 myocardial lesions were detected on PMMRI (chronic: 25; subacute: 16; acute: 30; and peracute: 53). Chronic, subacute, and acute infarction cases correlated very well to the myocardial findings obtained at autopsy. Peracute infarctions (age range: minutes to approximately 1h) were not visible on macroscopic autopsy. Peracute infarction areas detected on PMMRI could be verified in targeted histological investigations in 62.3% of cases and could be related to a matching coronary finding in 84.9% of cases. A total of 15.1% of peracute lesions on PMMRI lacked a matching coronary finding but presented with severe myocardial hypertrophy or cocaine intoxication facilitating a cardiac death without verifiable coronary stenosis.

PMMRI visualizes chronic, subacute, and acute myocardial infarction *in situ*. In peracute infarction as a possible cause of sudden cardiac death, it demonstrates affected myocardial areas not visible at autopsy. PMMRI can help to morphologically visualize so far invisibly affected ischemic myocardium postmortem and should be considered as a feasible postmortem investigation technique for sudden cardiac death cases.

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Sudden Cardiac Death, Postmortem MRI, Forensic Imaging

H43 Liver Laceration as a Complication of Cardiopulmonary Resuscitation (CPR)

Selcuk Cetin, MD, Gaziosmanpasa University Medical School, Dept of Forensic Medicine, Tokat, TURKEY; Hasan Din, MD*, Kayseri Branch of the Forensic Medicine Ministry, Kayseri, TURKEY; Murat S. Gürses, MD*, Uludag University School of Medicine, Dept of Forensic Medicine, Bursa, TURKEY; Filiz Eren, Council of Forensic Medicine, Morgue Dept, Bursa, TURKEY; Bulent Eren, Council of Forensic Medicine of Turkey, Bursa Morgue Dept, Bursa, TURKEY; and Eser Bayraktar, MD*, Uludag University School of Medicine, Dept of Forensic Medicine, Bursa, TURKEY*

After attending this presentation, attendees will have a better understanding of the potential complications involving resuscitation artifacts in patients, how to differentiate these injuries from trauma prior to resuscitation, and how to determine cause of death.

This presentation will impact the forensic science community by presenting a case of CPR injuries in a patient who had symptoms of myocardial infarction, diagnosed with echocardiography. These types of injuries, such as rib fractures, liver laceration, and contusions, are rare findings at postmortem examination. It is important that these injuries be recognized, differentiated from trauma prior to resuscitation attempts, and diagnosed as the main cause of death.

CPR is an emergency procedure performed in an effort to manually protect brain function until further measures are taken to return spontaneous blood circulation and breathing in a person who is in cardiac arrest.[1] CPR's complications associated with external cardiac compression include trauma to the heart and chest wall and gastrointestinal visceral injury including ruptured stomach and liver.[2] Liver abnormalities may contribute to an increased risk of laceration.[3] Thoracotomy ossification has caused a resuscitation laceration of the liver.[4] Meron et al. identified liver injuries in 0.6% of the patients who were non-traumatic in- or out-of-hospital cardiac arrests.[5]

This study presents a 43-year-old female whose cause of death was ischemic heart disease. She experienced a headache for the past two days and her father had died from heart disease. Except for these, there were no clinical findings. The hepatic injury findings were a surprise because of a lack of information about liver injuries or clinical signs evocative for those in the patient's medical file. CPR was performed for 45 minutes. The external examination at the autopsy revealed no pathologic findings. The internal examination at the autopsy revealed multiple injuries caused by the cardiac massage: liver laceration and contusion, bilateral rib fracture, massive intraperitoneal bleeding of 2,500cc. The heart weighed 306gr, coronary arteries showed occlusive atheroma plaques, and microscopic examination revealed perivascular fibrosis. Others organs revealed no pathologic findings for macroscopic and microscopic findings. The toxicological analysis revealed that no toxic agents or alcohol components were detected in the blood or urine specimens. This case brings attention to physicians of the issue of iatrogenic injuries following CPR and the possibility for these injuries to contribute to thanatogenesis.

This presentation will analyze the role of the pathologist in establishing the correct cause of death by being aware so findings are not misinterpreted.

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CPR, Artifacts, Liver Laceration

H44 A Nine-Year Review of All-Terrain Vehicle-Related Fatalities at the West Tennessee Regional Forensic Center: 2006–2014

Travis M. Sullivan, BS, 123 Harbor Club Circle, N, Apt 201, Memphis, TN 38103; and Zachary O'Neill, DO, West Tennessee Regional Forensic Center, 637 Poplar Avenue, Memphis, TN 38105*

The goal of this presentation is to present the epidemiology of All-Terrain Vehicle (ATV) -related fatalities reported to the West Tennessee Regional Forensic Center from 2006 through 2014.

This presentation will impact the forensic science community by describing the epidemiology of 35 ATV-related fatalities in order to assist local and state authorities in implementing preventative measures for ATV use.

According to the Consumer Product Safety Commission, more than 12,000 ATV-related fatalities have been reported in the United States between 1982 and 2012. The state of Tennessee, when compared with the other 49 states, accounted for the 7th highest number of reported ATV-related fatalities during this period. The primary purpose of this study was to present a retrospective review of ATV-related fatalities reported to the West Tennessee Regional Forensic Center (WTRFC) over a nine-year period from 2006 through 2014.

There were 35 cases of ATV-related fatalities reported to the WTRFC during this nine-year period. The yearly distribution of these 35 deaths ranged from no deaths in 2007 to eight deaths in 2010 (an average of 3.9 deaths/year). A majority of these fatalities were male (80.0%) and White (74.3%). The average age was 35.8 years (age range 1y-79y). The ATV accidents resulting in these fatalities occurred in Tennessee (20), Mississippi (9), Arkansas (5), and Missouri (1). Most of the accidents occurred on a roadway (60.0%), on the weekend (65.7%), and were roll-over accidents (42.9%). The decedent was the driver of the ATV in most cases (62.9%). In the majority of cases, 88.6% (31 of 35), the decedent was transported to a local or regional health care facility where they later died. In the other four cases, the decedent died at the scene of the accident. Injuries related to blunt force trauma were the primary cause of death in most cases (94.3%) while drowning was the cause of death in the other cases (5.7%). In 16 of the 35 cases (51.4%), toxicology was performed and the results were available for review. In 77.8% of these cases, toxicology detected some level of alcohol, common drugs of abuse, and/or selected medications. Although it was unknown if the decedent was wearing a helmet in most cases (21 of the 35 cases), where information was available, none of the 14 decedents were wearing a helmet.

All-Terrain Vehicle, West Tennessee, Fatalities

H45 Mechanisms of Death Due to Inadvertent Administration of Ionic Hypertonic Contrast Media Into the Subarachnoid Space

Kazuhiko Kibayashi, MD, Tokyo Women's Medical University, Dept of Legal Medicine, School of Medicine, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, JAPAN; Ryo Shimada, PhD, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, JAPAN; and Jiro Ezaki, MD, Tokyo Women's Medical University, 8-1, Shinjuku-ku 162-8666, JAPAN*

After attending this presentation, attendees will understand the mechanisms of death due to inadvertent administration of ionic hypertonic contrast media into the subarachnoid space.

This presentation will impact the forensic science community by demonstrating the mechanisms of death and pathological findings of inadvertent subarachnoid injection of ionic hypertonic contrast media. This presentation is also informative in preventing the misuse of contrast media.

Myelography is routinely performed using a non-ionic contrast media; however, the inadvertent administration of ionic hypertonic contrast media into the subarachnoid space results in convulsions and acute respiratory failure and can lead to death if not treated immediately. The mechanisms underlying the adverse effects of hypertonic contrast media on the central nervous system are unclear.

A literature review identified twelve surviving patients and five fatal patients after inadvertent administration of ionic hypertonic contrast media into the subarachnoid space (1971-2014). Most patients complained of lumbar or leg pain and developed convulsions within three hours of the administration. At autopsy, toxicological analysis of contrast media in the cerebrospinal fluid is required for the diagnosis of inadvertent administration.

The effects of a subarachnoid injection of hypertonic contrast media (60% Urografin®; osmotic pressure 6) on the central nervous system in rats were examined. Under general anesthesia, rats were administered a subarachnoid injection of 20.0μL, 10.0μL, 7.5μL, or 5.0μL of Urografin®, hypertonic sodium chloride solution (osmotic pressure 6), or saline. The rats that received 20.0μL or 10.0μL of Urografin® immediately developed severe convulsions and died within 42 minutes of the injection. The rats that received 7.5μL or 5.0μL of Urografin® exhibited delayed-onset convulsions that subsided within 240 minutes. Immunohistological examinations of the brain and spinal cord two days after the 7.5μL Urografin® injection revealed widespread microglial activation in the brain stem. Neither convulsions nor histological changes were observed in rats that received the hypertonic sodium chloride solution or saline injection. These findings indicate that the extent and duration of convulsions and fatality depend on the volume of hypertonic contrast media. Furthermore, brain stem injury due to the neurotoxicity of contrast media is the mechanism underlying the acute respiratory failure that occurs following the subarachnoid injection of hypertonic contrast media.

Contrast Media, Cerebrospinal Fluid, Medical Malpractice

H46 An Autopsy Case of Suspected Anti-N-Methyl-D-Aspartate Receptor (NMDAR) Encephalitis

Kino Hayashi, MD, Tokyo Medical Examiner's Office, 4-21-18, Otsuka, Bunkyo-ku, Tokyo 112-0012, JAPAN; Kumiko Asakura, MD, Tokyo Medical Examiner's Office, 4-21-18, Otsuka, Bukyo-ku, Tokyo, JAPAN; Wakako Hikiji, MD, Tokyo Medical Examiner's Office, 4-21-18, Ohtsuka, Bunkyo-ku, Tokyo, JAPAN; Tatsushige Fukunaga, MD, Tokyo Medical Examiner's Office, 4-21-18, Ohtsuka, Bunkyo-ku, Tokyo, JAPAN; Yohsuke Makino, MD, Dept of Forensic Medicine, The University of Tokyo, 7-3-1, Hongou, Bunkyo-ku, Tokyyo, JAPAN; Hisaomi Suzuki, MD, Shimofusa Psychiatric Medical Center, Chiba, JAPAN; Mitsumoto Onaya, MD, Shimofusa Psychiatric Medical Center, Chiba, JAPAN; and Takahiro Iizuka, MD, Neurology, Kitasato University, School of Medicine, Sagamihara, Kanagawa, JAPAN*

The goal of this presentation is to present a case concerning NMDAR encephalitis, which is one of the autoimmune limbic encephalitides.

This presentation will impact the forensic science community by explaining the manner in which forensic pathologists may perform an autopsy in a case of undiagnosed NMDAR encephalitis.

N-methyl-D-aspartate (NMDA) is a glutamate receptor and ion channel protein found in nerve cells, including those of the hippocampus. Anti-NMDAR encephalitis is one of the autoimmune limbic encephalitis. This report is of an autopsy case of suspected anti-NMDAR encephalitis, based on the patient's past psychiatric history and autopsy findings of bilateral hippocampal sclerosis and ovarian teratoma.

Introduction: Anti-NMDAR encephalitis is one of the limbic encephalitis associated with anti-NMDAR antibodies. It often presents as acute psychosis and is associated with paraneoplastic syndromes of ovarian teratomas in adult women. Acute autoimmune limbic encephalitis typically begins with a prodromal viral illness before the onset of psychiatric symptoms, seizures, and autonomic failure. This report is of an autopsy case of suspected anti-NMDAR encephalitis, based on the patient's past psychiatric history and autopsy findings of bilateral hippocampal sclerosis and ovarian teratoma.

Case Report: A 51-year-old woman was found dead in the bathtub. She worked as a part-time cashier and lived with her parents. She had been diagnosed with schizophrenia and had been undergoing treatment for the past three years. After dinner, she went to the bathroom to take a bath and 30 minutes later was found in an unconscious state, drowned, and soaked in water in the bathtub. She did not respond to resuscitation. The computerized tomography of her brain during resuscitation attempts was unremarkable. To determine the cause of death, the autopsy was performed 12 hours after her death.

Autopsy Report: The decedent was 154cm in height and weighed 56kg. No remarkable external findings of the body were present. On autopsy, macroscopic examination revealed water aspiration in the lungs and a 2.5cm right ovarian cyst. Microscopic examination revealed mature cystic teratoma of the right ovary and severe bilateral hippocampal sclerosis. Alcohol and drugs were not detected by toxicological analysis of the blood. The autopsy findings put the diagnosis of schizophrenia into question, and the patient's past medical history was reviewed.

Past History: Three years previously, the woman was a graphic designer and had no past medical history of seizure or psychiatric illness. Her family history was unremarkable. She was married at 27 years of age but divorced at 40 years of age because of financial issues with her husband. Before the onset, the patient had complained of being very tired, which was attributed to her daily hard work to manage a financial problem; however, one day she presented with acute onset of psychiatric symptoms characterized by confusion, memory loss, hallucinations, disorganized thinking, and incoherent speech. Two days later, she was examined at a local hospital but there were no abnormal findings on examination. On the following day, she was referred to a psychiatric hospital for further evaluation; however, during transfer to the psychiatric hospital, she developed a generalized seizure with high fever (38.1°C, 100.58°F) and was brought to the emergency department of a different hospital. Blood tests revealed leukocytosis (white blood cells 19,500/μL) with a normal serum C-reactive protein level. Cerebrospinal fluid examination revealed acellular spinal fluid (white blood cells, 6/μL) with normal protein and glucose levels. Brain computerized tomography was normal. The psychiatric symptoms were so severe that she was referred to a psychiatric hospital on the same day. On admission, she was found to have fever and hypoxia due to pneumonia, for which oxygen and medication were initiated. A few days later, although the patient was able to communicate, she remained disoriented with short-term memory loss and persistent delirium symptoms. She was subsequently diagnosed with schizophrenia and discharged one and one-half months after the onset. After discharge, she was referred to a local psychiatric clinic and prescribed only hypnotic drugs.

Conclusion: At the time of initial presentation, acute limbic encephalitis had not been suspected, and the patient had been diagnosed with schizophrenia; however, the autopsy findings and details of the initial clinical presentation strongly suggest that the patient had an acute limbic encephalopathy, such as anti-NMDAR encephalitis, rather than schizophrenia.

NMDAR Encephalitis, Hippocampal Sclerosis, Teratoma

H47 Medical Doctor Specialized in Legal Inspections (MDSLI): A Professional Interface Beween State Prosecutors and Medical Examiners — The Swiss Model

Emilio Scossa Baggi, via ferriere, Giubiasco, SWITZERLAND; Ilaria Monico, MS, Police Canton Ticino - Forensic Science Unit, via Chicherio 20, Bellinzona 6500, SWITZERLAND; Ario Conti, BD, Institute of Alpine Foundation for Life Sciences, 6718 Olivone, Olivone 6718, SWITZERLAND; Franco Ghiggia, MHME, Federazione Cantonale Ticinese Servizio Autoambula, Via Vergiò 8, 6942 Breganzona 6942, SWITZERLAND; Roberto Cianella, MHEM, Federazione Cantonale Ticinese Servizio Autoambula, Via Vergiò 8, 6942 Breganzona 6942, SWITZERLAND; Jhon Noseda, LLD, Ministero Pubblico Dello Stato e Repubblica del Ca, via Pretorio 6, Lugano, SWITZERLAND; Luisa Andrello, MD, Magenta Street 25/I, Olgiate Olona, Varese 21057, ITALY; Tony Fracasso, MD, PhD, CMU - CURML, Rue Michel-Servet 1, Geneva 1211, SWITZERLAND; and Patrice Mangin, MD, PhD, Centre Universitaire, Romand de Medecine Legale, Rue du Bugnon 21, Lausanne CH-1011, SWITZERLAND*

After attending this presentation, attendees will be aware of a new model of organization established in Swiss Canton Ticino for cases of death due to unnatural causes.

This presentation will impact the forensic science community by demonstrating the possibility of a professional interface between state prosecutors and medical examiners with the goal of improving the quality of work and data at the time a body is discovered.

In recent years, the Swiss Confederation decided to revise the Swiss Criminal Procedure Code published on October 5, 2007, in effect by January 1, 2011.1 Regarding the procedure related to the observation of death in the case of death by unnatural causes (see the circumstances of death or serious event of unknown origin) the new version of the Swiss Criminal Procedure Code clearly defines who will be responsible for this task. Specifically, Section 6, Article 253, Examination of Dead Bodies, draws attention to the following points. First, if there are indications that a death is not due to natural causes, but, in particular, is due to a crime, an accident, or a suicide or if the identity of the body is unknown, the public prosecutor arranges for inspection by a specialized doctor in order to either clarify the cause of death or identify the body. Second, if after the legal inspection of the body there is no evidence of a crime and the identity of the decedent has been established, the prosecutor delivers the body and authorizes the funeral. Otherwise, the prosecutor gives orders for the body to be kept in a safe place and requires additional inspections by the Institute of Legal/Forensic Medicine (i.e., autopsy). The prosecutor may also order that the entire body, or part of it, be retained for the purpose of investigation. Last, Swiss Cantons determine which medical personnel members are obliged to announce to the criminal justice authorities those deaths that are due to suspicious or unknown causes.

Starting from this legal basis, the Canton Ticino had to adapt to what was foreseen of the federal law; however, unlike the Swiss Cantons that have their own Institute of Forensic Medicine — normally linked or associated to a university — the Canton Ticino region has no such structure. The non-realization or absence of an Institute of Legal Medicine in this Italian part of Switzerland (i.e., Canton Ticino) has partially penalized but also stimulated creativity in finding solutions to ensure quality service for justice and for the search for truth in such a complex and interdisciplinary area such as that of forensic science. In 2012, the state prosecutor and the State of Canton Ticino, in a strict collaboration with the University Centre of Legal Medicine Lausanne-Geneva (CURML), have enabled the realization of an important and innovative service equal to the one offered by the Medical Doctors Specialized in Legal Inspections (MDSLI). The Swiss Society of Legal Medicine (SSML) 2015 summer meeting provided the opportunity to inform professionals and experts of the establishment of a new professional figure, that of MDSLI, based on the requirements of the Swiss code of criminal procedure. It must be immediately specified that the role and competence of MDSLI differ from the one of the medical examiner. The MDSLI doctor is solely responsible for carrying out observations and for certifying the death through an external medical examination of the decedent body at the place where the body has been discovered. MDSLI must take place at the scene of the crime in collaboration with any other on-scene investigator and acts as a liaison between the public prosecutor and the police on one side and the legal doctor/coroner on the other side. This doctor performs his/her functions according to the expert's mandate that was received from the public ministry and that result in the certificate of death in a case of natural death or of a legal inspection report in a case of suspicious, doubtful, or undetermined (i.e., non-natural) death.

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Medical Examiner, Coroner, Switzerland

H48 Expression of Heat Shock Protein 70 (HSP70) After Human Brain Injury in Different Post-Traumatic Intervals

Martina Focardi, Largo Brambilla 3, Florence 50134, ITALY; Vilma Pinchi, PhD, via Della Resistenza 14, Murlo, Siena 53016, ITALY; Defraia Beatrice, Largo Brambilla 3, Florence, ITALY; Laura Pieri, Via delle Gore 2/E, Florence 50134, ITALY; Francesca Castiglione, Largo Brambilla, Florence 50134, ITALY; and Gian A. Norelli, sez.dep.Medicina Legale, Firenze, ITALY*

After attending this presentation, attendees will better understand immunoistochemestry as it applies to blunt trauma.

This presentation will impact the forensic science community by increasing knowledge about brain injury and its correlation with the expression of HSP70, which is an important issue in forensic cases.

Traumatic Brain Injury (TBI) is a very frequent cause of death after traffic accidents or assaults. The response to TBI is complex and induces various biological pathways in all brain regions that contribute to bad outcomes.[1] TBI frequently leads to brain edema and hemorrhage due to disruption of the Blood Brain Barrier (BBB). This promotes the neurotoxic cascade such as the energy-dependent ion pumps failure, acidosis, membrane depolarization, the influx of calcium and sodium, the release of glutamate, and the activation of apoptosis and inflammation.[1] The inflammatory response, after TBI, involve microglial activation, leukocyte recruitment, and upregulation of cytokine secretion.[2]

The Heat Shock Proteins (HSPS) comprise a highly conserved family of Adenosine Triphosphate (ATP) -dependent, cytosolic chaperones that function primarily in facilitating protein folding, degradation, complex assembly, and translocation, consequently preventing harmful protein aggregation.[3] Heat shock proteins are induced by many stressful stimuli, including a variety of central nervous system insults, such as cerebral ischemia, neurotoxin exposure, and when normal cellular processes are interrupted by stress.[4] The 70kDa inducible HSP (HSP70) is increased in brain vessels following experimental TBI and its induction can protect against a variety of insults including brain ischemia, trauma, and hemorrhage.[5-8] Finally, a human study indicates that the highest expression of HSP70 is found at 0h after brain contusion; the intensity of HSP70 staining decreases to the minimum at 24h after TBI, then increases gradually.[9]

The goal of this research is to assess the different expression of HSP70 immunohistochemically and molecularly in individuals with different posttraumatic intervals. This data will allow this study to determine if there is a linear relationship between the expression of this protein and the time of survival.

This study includes the reference group (control) and the TBI group. In the reference group (n=30), there were no brain contusions and death had occurred within 0-30min as a result of other reasons (myocardial infarction, pulmonary embolism, ruptured aneurysm). The TBI group (n=30) comprised individuals with a frontal cortical contusion zone and cranial traumata as the cause of death. The survival time in the TBI group varied between 0-30min, 30min-2h, 4h-12h, 24h-48h. The causes of injury in the TBI group were falls, household accidents, and vehicle accidents.

In each group, samples of brain tissue (middle-brain and brain parenchyma) were fixed with 10% formaldehyde for more than one week and embedded in paraffin; four to five micron-thick sections were stained with hematoxylin and eosin and by immunohistochemistry for HSP70 antigen.

A preliminary analysis, after hematoxylin and eosin staining, performed on the control group and on the TBI group (0min-30min), showed no difference in the distribution of cerebral edema. Edema thus is not dependent on the factor that induces stress (anoxia, trauma, etc.). The preliminary investigation of immunohistochemistry has shown a correlation between the application time of the trauma and the expression of HSP70.

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Brain, Injury, HSP70

H49 Computed Tomography (CT) Findings of Unsuspected Aortic Dissection and Adult Polycystic Kidney Disease (APKD)

Christopher J. Gordon, MD, AFMES, 115 Purple Heart Drive, Dover AFB, DE 19902; Edward Mazuchowski II, MD, PhD, 115 Purple Heart Drive, Dover AFB, DE 19902; Wendy S. Warren, DO*, Armed Forces Medical Examiner System, 115 Purple Heart Drive, Dover AFB, DE 19902; and Howard T. Harcke, Jr., MD, 3205 Coachman Road, Wilmington, DE 19803*

The goal of this presentation is to assist attendees in identifying the postmortem CT findings which alert the forensic pathologist to the coexistence of acute aortic dissection and APKD.

This presentation will impact the forensic science community by assisting those performing medicolegal death investigations to recognize the association of aortic dissection and APKD, enabling them to use these findings on postmortem CT to enhance the performance of the autopsy.

Postmortem CT has been used during medicolegal death investigations to depict pathologic findings prior to the autopsy procedure. Use of CT in deaths by natural causes is more difficult than those associated with ballistic injury and trauma because pathology involves differentiation of soft tissue which is limited without the use of contrast. Patterns which are recognizable can be used to prompt the search for particular pathology. Two cases of unsuspected APKD illustrate this.

Case 1: A 39-year-old male who was admitted to the hospital for treatment of deep vein thrombosis and pulmonary emboli became acutely unresponsive and died despite medical intervention. Postmortem CT showed irregular contours and cystic changes in enlarged kidneys and diffuse retroperitoneal hemorrhage in addition to pulmonary congestion. Margins of the abdominal aorta were obscured. Autopsy revealed the presence of pulmonary emboli and confirmed the CT findings of cystic kidneys and diffuse retroperitoneal hemorrhage. A 1cm full-thickness defect was present on the posterior abdominal aorta approximately 11cm proximal to the renal arteries. Histologic sections from the defect showed transmural separation and dissecting hemorrhage through layers of the tunica media. The liver was noted to show no definitive cysts.

Case 2: A 62-year-old male was found unresponsive in his home after feeling ill and receiving treatment for gastrointestinal complaints. Postmortem CT showed massive kidneys composed of multiple cysts of varying size and density. Areas of the liver suggested cysts. The heart was enlarged and this was noted on the CT to relate to a large pericardial effusion. The ascending aorta was widened and had irregular margins. At autopsy, a 950mL bloody pericardial effusion was noted. At the aortic valve annulus, there was a longitudinal intimal tear joining a transverse intimal tear with accompanying false lumen. Histologic sections demonstrated a split in the media with layered deposition of blood elements, degenerative changes in the media, and mixed inflammation. The liver contained multiple scattered cysts measuring up to 2.5cm.

Adults with APKD are known to be at risk for vascular abnormalities. The most widely recognized is the intracranial or berry aneurysm. Aortic dissection is also reported and its postmortem CT association is typically pericardial effusion. Case 2 matches this pattern and the laceration is in the arch of the aorta. In Case 1, the CT findings are manifest below the diaphragm and relate to retroperitoneal hemorrhage. This correlates with the location of the laceration in the descending aorta at the level of the diaphragm. There are multiple conditions in which aortic dissection can occur and the suspected mechanism seems to relate to hypertension and/or tissue abnormality. The association with APKD invokes both of these as possibilities. Since APKD is known to affect the liver, the postmortem CT should be examined for involvement. Liver visualization in both cases was suboptimal because the scans were performed with the arms at the sides which introduced artifacts. Correlation between CT and autopsy was correct in both cases with respect to presence but not with regard to details of size and number. In cases with APKD, scans should be repeated with the arms raised overhead for optimal assessment of abdominal pathology.

The pathologist discovering polycystic kidneys on postmortem CT should look for hemorrhage and use the pattern to guide the search for aortic dissection.

Polycystic Kidney, Aortic Dissection, APKD

H50 Pack Mentality: Fatal Mauling in the African Painted Dogs' Zoo Exhibit

Farshaad Bilimoria, MD, 10 Allegheny Center, Apt 224, Pittsburgh, PA 15212; Stacey L. Reed, DO, 9500 Babcock Boulevard, Apt 54, Allison Park, PA 15101; Jessica B. Dwyer, MD, Allegheny County Medical Examiner's Office, 1520 Penn Avenue, Pittsburgh, PA 15222; Joseph A. DelTondo, DO, West Virginia University, One Medical Center Drive, Dept of Pathology - PO Box 9203, Morgantown, WV 26506; Todd M. Luckasevic, DO, Alleghey County ME, 1520 Penn Avenue, Pittsburgh, PA 15222; and Karl E. Williams, MD, Allegheny County OME, 1520 Penn Avenue, Pittsburgh, PA 15222*

The goal of this presentation is to illustrate a rare and avoidable death involving a member of the public accidently falling into a zoo exhibit housing a pack of endangered African painted dogs.

This presentation will impact the forensic science community by illustrating the injury patterns of a pack of wild hypercarnivorous dogs and the need for proper zoo barriers and safe viewing platforms.

African painted dogs (*Lycaon pictus*) are among the most critically endangered species with roughly 5,000 dogs currently remaining in the wild. These animals are nomadic hunters and require expansive home ranges. Their method of pack hunting involves exhausting prey through constant pursuit before tackling their target. In turn, pack hunting produces a unique pattern of injuries. The head, neck, and extremities display shallow lacerations and puncture wounds obtained during the chase, while the trunk displays traumatic evisceration from the actual feasting. This method is considered highly successful, with approximately 80% of hunts resulting in a kill. In addition, bite force studies have ranked the African painted dog bite as one of the strongest at 719 Pascal, second only to the grey wolf.

A local zoo housed a pack of 11 African painted dogs. The dogs had no direct human contact, and their training was limited to retreating to their kennels when a whistle was blown. A viewing platform above the pen allowed the general public to observe the dogs. The wooden platform had five sides with a four foot-high railing. The two posterior-lateral walls had acrylic glass barriers, the two anterior-lateral walls had chain-link fencing, and the anterior wall was left open.

This case involves a two-year-old male visiting the zoo with his family. The group entered the African painted dogs' viewing platform. The mother lifted the decedent so he could stand on the railing and lean against one of the acrylic glass barriers. Eventually, the mother brought the decedent to the anterior wall and placed him on the rail, assuming there was a acrylic glass barrier. Unfortunately, the mother let go, the decedent leaned forward as if to place his hands on acrylic glass, and fell forward 11'4", first onto a safety net and then into the dogs' pen. The decedent was witnessed standing up before immediately being attacked by the 11 dogs. A trainer responded to witnesses' screams and blew a whistle. Seven of the dogs ran back to their kennels. A zoo response team shot tranquilizer darts at the remaining dogs, scaring three additional dogs back to the kennels. Responding officers arrived and opened fire on the last dog, who remained by the decedent. Medical teams arrived and pronounced the decedent without resuscitation attempts.

As per the chief veterinarian of the zoo, the dogs were regularly fed within their individual kennels, except once a week when they were provided a deer carcass to consume as a pack. They were scheduled to have their weekly pack meal on the day the decedent visited, but had not yet received the meal.

Examination of the decedent's body showed numerous blunt and sharp force injuries. Over 220 contusions, abrasions, and lacerations were identified on the head, neck, trunk, and extremities. Multiple two-patterned puncture wounds were noted on the head and chest. No internal craniocervical trauma was noted. There were extensive defects of the skin, subcutaneous tissues, muscles, and bones of the trunk and thighs, as well as traumatic evisceration of all internal organs of the chest, abdomen, and pelvis. Field recovery produced a 30 gram portion of lung tissue, a 60 gram portion of liver, and two portions of intestine, measuring 180cm and 104cm each. Examination of the dog killed by the police showed blood on the face, teeth, and left front paw. Gunshot wounds were identified to the head, back, and right hind leg, with bullet fragments recovered from each location. Internal examination revealed the dog's esophagus and stomach to be empty.

Fatal attacks by animals held in captivity are rare and typically involve zoo employees or animal handlers. This case underscores the importance of facilities housing wild animals to develop safe viewing options for the general public and for the public to adhere to advertised safety procedures. The characteristic injuries documented in this witnessed attack will hopefully help the forensic community identify cases of unwitnessed pack attacks.

African Painted Dogs, Zoo, Mauling

H51 A Fatal Moose Attack

Petur G. Gudmannsson, MD, Rättsmedicinalverket, Artillerigatan 12, 58758, Linköping, Östergötland 58758, SWEDEN; Anders Eriksson, MD, PhD*, Umea University, Dept Forensic Medicine, PO Box 7616, Umea SE-907 12, SWEDEN; Johan Berge, MD, Rättsmedicinalverket, Artillerigatan 12, Linköping 58758, SWEDEN; Henrik Druid, MD, PhD, Karolinska Institutet, Dept of Forensic Medicine, Retzius v. 3, Stockholm SE-171 77, SWEDEN; and Göran Ericsson, Swedish University of Agricultural Sciences, Skogsmarksgränd, Umeå 90183, SWEDEN*

After attending this presentation, attendees will appreciate the possibility of a fatal moose attack when encountering a dead body in a boreal area. The value of utilization of experts from other fields of study, prudency regarding scene integrity, and thoughtful sampling for trace material is also illustrated.

This presentation will impact the forensic science community by reporting an unexpected cause of death, that of a fatal moose attack, when investigating a traumatic death in a boreal area.

Fatal animal attacks are uncommon. In the United States, the most common non-venomous species involved is dogs; however, fatal moose attacks are extremely rare, with only one case previously reported in the scientific literature.[1,2]

During the early fall in rural Sweden, a woman in her sixties was walking her dog in the woods. When she failed to return home in the evening, her spouse went looking for her, only to find her severely injured dead body by a small lake in the forest, lying beside a row boat. Her body exhibited widespread, severe blunt trauma, including a conspicuous, deep laceration on the right leg, and her jeans were torn from the waist down. Still, only a small amount of blood was initially found at the scene.

A homicide investigation was started and initially the scene was regarded as a dumping site rather than a crime scene. The autopsy revealed grass tucked deep into some of the large wounds. Severe thoracic injuries with flail chest was found to be the cause of death. At the outset, the victim was assumed to have been run over with the prime suspect's riding lawn mower; however, reconstruction with the lawn mower and results from the initial forensic investigation provided no support to this theory.

Later, a bloodstain pattern analysis on the boat revealed that the victim had been struck numerous times at the scene, inspiring alternative theories as to the mode of death, primarily featuring a possible animal attack. The views of a wildlife specialist at the Swedish University of Agricultural Sciences were sought, leading to subsequent revision of the case and further sampling. This resulted in a turning point in the investigation, owing to trace material from the victim and her clothes that was later recovered and proved to originate from a moose, specifically saliva and hair; however, confirming moose tracks at the scene was not possible due to rain and disruption by first responders.

It was finally concluded that the woman's dog likely provoked a moose, catalyzing an attack that ultimately targeted the woman and included goring as well as trampling.

The pattern of injury in this case resembles those previously reported from attacks by cattle.[3,4] Fatal moose attacks constitute an extremely rare threat in boreal areas, but can be considered in traumatic deaths of unknown cause.

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Animal Attack, Moose, Boreal Death

H52 Fatality During Servicing of a Fire Extinguisher: A Case Study

Nilesh K. Tumram, MD, 85 Anantnagar, Nagpur, Maharashtra 440013, INDIA*

After attending this presentation, attendees will be able to evaluate a fatality caused by a fire extinguisher brought in for service by maintenance personnel.

This presentation will impact the forensic science community by creating awareness that there is the very real possibility that a cartridge-operated fire extinguisher that hasn't been properly maintained could explode upon activation, causing serious injury to the operator or bystanders. This presentation highlights the fact that such fire extinguisher cylinders do explode and can cause fatal injuries.

A fire extinguisher, or extinguisher, is an active fire protection device used to extinguish or control small fires. Usually, a fire extinguisher consists of a hand-held cylindrical pressure vessel containing an agent which can be discharged to extinguish a fire. The two main types of fire extinguishers are stored pressure and cartridge-operated. In stored pressure units, the expellant is stored in the same chamber as the fire-fighting agent itself. A cartridge-operated extinguisher consists of a cylinder filled with the extinguishant (water, foam, powder, etc.) and a gas cartridge containing highly pressurized Carbon Dioxide (CO_2). The pressure in the cylinder is only released from the cartridge once the handle is squeezed and pierces the cartridge, which will drive the extinguishant out of the cylinder via the hose.

Stored pressure extinguishers consist of a cylinder containing the extinguishing agent (water, powder, foam, etc.) and are permanently pressurized with either dry air or oxygen-free nitrogen. On activation by squeezing the handle, the valve of extinguisher inside releases and the pressure pushes the extinguishing agent out through the hose. Cartridge-operated extinguishers have an advantage over their stored pressure counterparts in that their outer cylinder can be pierced without the extinguisher exploding. If a stored pressure extinguisher cylinder is pierced, it would release the pressure explosively.

During basic maintenance service on a cartridge-operated extinguisher, most of the steps are the same as those taken when servicing a stored pressure extinguisher. The additional steps involve: (1) carefully unscrewing the extinguisher's head cap (as sometimes the cartridge may have been pierced or leaked inside the cylinder); (2) removing and examining the cartridge, which includes weighing the cartridge to ensure it has not lost more than 10% of its original weight; (3) checking to ensure it is still within its ten-year lifespan from date of manufacture; (4) checking the head cap mechanism to ensure that when the handle is squeezed, the sharp spike that should pierce the cartridge is in good condition and functional; (5) pouring out the extinguishing agent (in the case of water-based agents such as water and foam; the powder extinguishing agent is checked by covering the neck of the cylinder and inverting it to ensure the content moves freely); and, (6) examining the interior of the cylinder to ensure there is no corrosion or flaking of the cylinder's Polyvinyl Chloride (PVC) coating (for water and foam).

The rationale for these additional steps is to ensure that the cylinder is capable of absorbing an instant violent pressurization such as occurs when the CO_2 cartridge is pierced as the extinguisher is being used on a fire. A weak cylinder could be catastrophic if it could not maintain integrity due to corrosion or any other internal damage and could explode upon activation, causing serious injury to the operator or bystanders.

Human Fatality, Fire Extinguisher, Investigation

H53 Getting It Right: How Seemingly Obvious Manners of Death Can Change Through Historical and Autopsy Investigations

Phoutthasone Thirakul, MD, 16619 Palm Royal Drive, #238, Tampa, FL 33647; Daniel L. Schultz, MD*, Lifelink Tissue Bank, 9661 Delaney Creek Boulevard, Tampa, FL 33619; and Kelly G. Devers, MD, Hillsborough County Medical Examiner, 11025 N 46th Street, Tampa, FL 33617*

After attending this presentation, attendees will understand the value of manner-of-death classification and how a variety of factors can affect that seemingly obvious determination.

This presentation will impact the forensic science community by elucidating the process of how medical examiners arrive at a manner of death. Participants will understand the importance of detailed history gathering and corroboration with physical findings, as well as that of the detailed autopsy in corroborating or refuting trauma. Measuring the impact of a correct/incorrect manner determination on society and on potential living "victims" of incorrect calls is part of the mission of the forensic pathologist.

Recording the manner of death is necessary for all death certificates in the United States. There is frequently great debate in how various deaths are certified. It is important to gather as much historical and direct observational data as possible to make that opinion. Although history is a vital component of manner determinations, the value of autopsy cannot be underestimated in confirming or refuting manners such as homicides, suicides, and even apparent natural deaths, which can have significant societal impact, emotionally, financially, and for future health and safety considerations.

Two cases are presented that show opposite ends of the spectrum. One demonstrates the value of knowing the proximate cause/circumstances leading to death that may not initially be known or reported, while the other shows the value of autopsy in spite of compelling historical circumstances. The first, an apparent natural vs. accident-turned-homicide in an individual with chronic renal failure and dialysis (port rupture), was subsequently determined to be a delayed homicide due to remote gunshot wound requiring blood transfusion, leading to hepatitis C, liver transplant, post immunosuppressive renal failure, dialysis, and subsequent port dislodgment and exsanguination. The other was an apparent delayed homicide with no drug abuse history reported, which, based on the autopsy examination, revealed evidence of chronic intravenous drug abuse with pulmonary methyl cellulose granulomatosis (via chronic indwelling port), as well as cor pulmonale in the company of *Staphylococcus aureus* sepsis and terminal sudden cardiac event. His apparent homicidal manner was subsequently changed to natural (chronic intravenous drug abuse). The history and the autopsy are not mutually exclusive, and sometimes history or the autopsy make the more compelling argument in manner determinations.

Manner, Death, Autopsy

H54 Diagnostic Accuracy of Postmortem Imaging vs. Autopsy: A Systematic Literature Review

Anders Eriksson, MD, PhD, Umea University, Dept Forensic Medicine, PO Box 7616, Umea SE-907 12, SWEDEN; Torfinn Gustafsson, BM, Section of Forensic Medicine, Umeå University, PO Box 7616, Umeå SE-907 12 Umeå, SWEDEN; Monica Hultcrantz, PhD, Swedish Agency for Health Technology Assessment, PO Box 3657, Stockholm, SWEDEN; Malin Höistad, PhD, Swedish Agency for Health Technology Assessment, PO Box 3657, Stockholm SE-103 59, SWEDEN; Stella Jacobson, PhD, Swedish Agency for Health Technology Assessment, PO Box 3657, Stockholm SE-103 59 Stockholm, SWEDEN; and Anders Persson, MD, PhD, CMIV, Linköpings Universitet/US, Linköping 581 85, SWEDEN*

After attending this presentation, attendees will have a better perception of the scientific evidence, or lack thereof, for substituting the autopsy with postmortem imaging, a better understanding of the problems with published research, and more information concerning what is needed to enhance this level of knowledge.

This presentation will impact the forensic science community by providing information concerning the lack of scientific evidence for the diagnostic accuracy of postmortem imaging and by describing how to help focus future research.

The autopsy has long been regarded as the "gold standard" for retrospective quality assessment of clinical diagnoses; comparisons of clinical diagnoses and autopsy findings have revealed major discrepancies in 25% or more in deceased, autopsied patients; however, during the past 50 years, clinical autopsy rates have drastically declined in the Western world.[1,2] The reasons for this are manifold and include advances in laboratory diagnostic technology and imaging techniques that often — wrongfully — result in the belief among clinicians that the autopsy has become redundant.

A low autopsy rate may conceal medical malpractice and thereby prevent an important quality assurance indicator in health care. The reliability of the cause-of-death statistics decreases and the family may be given wrongful or insufficient information. Hence, the decrease in the clinical autopsy rate has negative consequences for the family, for future patients, for health care, and for society as a whole.

Postmortem imaging has emerged as a possible alternative to compensate for this continuous decline in clinical autopsies. In medicolegal autopsies, postmortem imaging has been used for more than a century as a complement; however, the diagnostic accuracy of postmortem imaging for various types of findings was not previously systematically analyzed and this is the focus of the present literature review.

A literature search was performed in the databases PubMed®, EMBASE®, and Cochrane Library through January 7, 2015. Only published studies in English and with ten or more individuals were accepted if the results were presented on an individual level. The criteria for eligibility included population studies on deceased individuals of any age in a forensic or clinical setting; index test studies on diagnostic imaging techniques used in clinical practice today (Computed Tomography (CT), Magnetic Resonance Imaging (MRI), ultrasound, and conventional X-ray techniques); reference test autopsy and/or histopathological examination; and outcome measure diagnostic accuracy of a specific finding expressed as *sensitivity and specificity*, or *agreement and discrepancy*.

All studies of potential relevance according to the inclusion criteria were obtained in full text and two reviewers independently assessed them for inclusion. The relevant publications were assessed for risk of bias using the QUADAS tool and classified into having low, moderate, or high risk of bias according to defined criteria.[3]

The search generated 2,600 abstracts, of which 340 were assessed as possibly relevant and read in full text. After further evaluation, 71 studies were included in total, 49 assessed as having high risk of bias and 22 as having moderate risk of bias.

In general, based on the current scientific literature, it is not possible to determine the diagnostic accuracy of postmortem imaging in conjunction with, or as alternative to, autopsy. The reason for this is that the included studies have investigated different populations, used different techniques, and analyzed and presented the results in different ways. Hence, the results from different studies cannot be weighed together; however, Individual studies can indicate for what findings the techniques might be useful (e.g., for determining organ weights) and that imaging techniques are superior to autopsy in detecting gas.

To correctly determine the usefulness of postmortem imaging, future studies need improved planning, higher quality, and larger materials. Cooperation in multicenter studies could be one way to proceed.

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Postmortem Imaging, Diagnostic Accuracy, Autopsy

H55 Prevalence and Etiology of Intervention-Related Deaths — A Swedish Perspective

Torfinn Gustafsson, BM, Umea University, Dept Forensic Medicine PO Box 7616, Umeå, Västerbotten 907 12, SWEDEN; Peter Carlsson, MD, Sect of Forenisc Medicine Umeå University, Umeå 907 12, SWEDEN; Fredrik Tamsen, MD, MSc, The Swedish National Board of Forensic Medicine, Rättsmedicinalverket, Box 1024, Uppsala 751 40, SWEDEN; and Anders Eriksson, MD, PhD, Umea University, Dept Forensic Medicine, PO Box 7616, Umea SE-907 12, SWEDEN*

After attending this presentation, attendees will understand the etiology and prevalence of intervention-related deaths in Sweden.

This presentation will impact the forensic science community by providing information on prevalence, etiology, and associations of intervention-related deaths in a northern European population.

Deaths related to intervention by police are often highly publicized and often lead to accusations of the forensic and legal system covering up police brutality, leading to a risk of undermining the public's trust. Knowledge of risk factors, circumstances, and mechanisms related to these fatalities is therefore of value.

Two main mechanisms have been discussed in relation to sudden death during intervention: Excited Delirium Syndrome (EDS) and positional asphyxia. EDS is characterized as a life-threatening condition where the decedents expresses bizarre and violent behavior, hyperthermia, and sudden death, mostly following a violent restraint situation. The syndrome has a strong link to drug use, primarily cocaine and methamphetamine.[1,2] Positional asphyxia refers to a situation where the decedent has been put in a body position that restrains breathing that he or she could not escape. This is often combined with so-called traumatic asphyxia, a situation in which there is a heavy pressure exerted on the thorax, preventing normal breathing. The most common body position described is a prone position with arms and legs tied behind his or her back, often referred to as a "hog-tie" position.[2]

All deaths linked to intervention by the police and other authorities with direct or short-range contact from 1992 through 2014 were collected from the Swedish National Board of Forensic Medicine data base. The study included all deaths that could be directly linked with a physical intervention or shooting and where loss of consciousness or death occurred within ten minutes of the encounter.

During the study period, there were 22 shooting deaths and 33 non-shooting deaths, making an annual average of 0.010 and 0.016 deaths/100,000 citizens, respectively. There was a decline in the incidence of non-shooting deaths, 0.024 and 0.008/100,000 citizens during 1992-2003 and 2004-2014, respectively. The corresponding figures for shooting deaths were 0.009 and 0.012, respectively.

In the shooting-death population, all decedents were male with a mean age of 36.6 years. Eighteen (82%) were under the influence of alcohol and/or drugs, most commonly amphetamine. In most cases, there was a perceived threat by the police, the most common threat being the decent attacking with a knife. The majority of shots fired by the police were at short range and without prior warning shots (59%).

In 20 (61%) non-shooting deaths, excited delirium syndrome was deemed the probable cause of death. Of the remaining 13 cases, the cause of death was traumatic asphyxiation in five cases (38%), aspiration of stomach contents in two cases (15%), cardiac arrhythmia in one case (8%), and undetermined in five cases (38%). In the EDS group, 17 were male and 3 female with a mean age of 37.4 years. The decedents in this group were all under the influence of drugs (80%) and/or showed psychotic behavior prior to death. Police were involved in 22 cases (67%) overall and in 18 of the EDS cases (90%).

Police are involved in the majority of these deaths, especially EDS cases; however, intervention-related deaths are rare and, compared to the number of people detained for intoxication in Sweden, there was roughly one death per 31,000 such events during the past ten years. The true rate of death per police intervention is probably far less.

The majority of decedents in Swedish intervention-related deaths are males in mid to late 30s with either significant comorbidity and/or drug intoxication, the most common intoxicating agent being amphetamine.

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Excited Delirium Syndrome, Police Intervention, Police Shootings

H56 Treatment for Injury Predicts the Risk of Child Homicide — A Case-Control Study

Björn Bäckström, National Board of Forensic Medicine, Box 7616, Umeå, AC 90712, SWEDEN; Jonatan Hedlund, MD, Department of Clinical Neuroscience, Karolinska Institutet, PO Box 4044, Huddinge 141 04, SWEDEN; Anna Jinghede, DDS, Department of Clinical Neuroscience, Karolinska Institutet, Huddinge 141 04, SWEDEN; and Joakim Sturup, PhD, Department of Clinical Neuroscience, Karolinska Institutet, PO Box 4044, Huddinge 141 04, SWEDEN*

After attending this presentation, attendees will better understand how children at risk of becoming victims of child homicide potentially can be identified.

This presentation will impact the forensic science community by increasing awareness of victim risk factors for child homicide.

There has been a decrease in child homicides in Sweden from eight to ten victims annually in the early 1990s to four to five victims in the late 2000s.[1] The majority of previous research on risk factors for child homicide has focused on risk factors regarding perpetrators (e.g., mental illness or drug abuse). A recent Swedish study reports that multiple birth (i.e., being a twin) is a risk factor for becoming a victim of child homicide.[2] Thus, the goal of this study was to determine whether previous medical care for injuries and/or intoxication is a risk factor for becoming a victim of intra-familial child homicide.

This study had a case-control design. All child homicide victims (0-14 years) in Sweden from 1994 to 2012 were identified in a database for autopsies administrated by the National Board of Forensic Medicine. Extra-familial cases and children who died within 24 hours after birth were excluded, leaving a total of 74 study cases. Autopsy reports, police reports, and court verdicts were studied. Five controls, matched for age, sex, and geographical proximity, were collected for each case through Statistics Sweden, resulting in a total of 370 controls. Data on previous health care use due to injuries were collected from the Swedish National Patient Registry.

The cases were more likely than the controls to have undergone previous treatment for injuries (inpatient and outpatient care, Odds Ratio (OR)=2.3; 95% Confidence Interval (CI) 1.1-4.7). The results were even more pronounced when looking only at inpatient care (OR=6.2; 95% CI 2.2-17.9). The difference was driven mainly by higher odds for female victims with an OR of 3.9 (95% CI 1.3-11.7) for any medical intervention and 9.7 (95% CI 2.2-42.5) for inpatient care. The same trend, although not statistically significant, could be identified for boys regarding inpatient care ($p=0.065$; $\chi 2=3.41$) but not for overall treatment. In most cases, the injuries were not identified by the clinicians as being caused by abuse.

In conclusion, health care clinicians should be aware that children who are treated for injuries, even when not suspected to be by abuse, are at elevated risk for violent victimization. It is reasonable to say that the study supports the Swedish legislation that states clinicians must alert the social services whenever a child is deemed to be at risk of victimization; however, due to the low base rates of homicide victimization, there is an obvious risk that parents and families are stigmatized. Hence, issues of child abuse and reports to the social services need to be handled in a clinically, scientifically, and judicially sound way.

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Child Homicide, Previous Injuries, Filicide

H57 Deaths Associated With Choking: An Istanbul Experience

Erdinc Ozdemir, The Council of Forensic Medicine, Mus Courthouse, Forensic Medicine, Mus, TURKEY; Muhammet Nabi Kantarci, Council of Forensic Medicine, Kimiz sk. Çobançesme Mh, Bahçelievler, Istanbul, TURKEY; Timucin Yildirim, Istanbul Adli Tip Kurumu Baskanligi Yenibosna, Bahçelievler/Istanbul, Istanbul, TURKEY; and Sermet Koc, Adli Tip Kurumu Morg Dairesi Baskanligi, Bahcelievler, Istanbul 34196, TURKEY*

After attending this presentation, attendees will have insight into the effects and causes of choking and points to consider during an autopsy.

This presentation will impact the forensic science community by providing a six-year retrospective study from Istanbul describing choking-related deaths.

Choking-related death is a kind of death which occurs when food or foreign bodies occlude the respiratory tract. In an autopsy examination of choking-related deaths, the presence of a foreign body in the respiratory tract and general findings of asphyxia are helpful in the diagnosis, when another reason that explains the cause of death cannot be detected. There are many risk factors that provide a tendency toward choking. These include the habit of swallowing large amounts of food, bad/missing teeth, alcohol consumption, cerebrovascular diseases, dementia, Parkinson's disease, schizophrenia, sedatives, antipsychotic drug use, etc.

Asphyxia related to foods is classified as "acute accidental death." The mode of death that occurs secondary to the obstruction of the upper respiratory tract while eating in a restaurant has previously been named "café coronary." Corpophagic café coronary and therapy-related café coronary, which are rare forms, have also been reported in the literature.

In this study, the goal was to learn if these types of deaths are preventable by revealing demographic features and predisposing factors of these cases. In addition, the goal was to investigate the presence of some specific features, especially by evaluating the tracheal findings at autopsy.

In this study, a total number of 30,221 forensic autopsies performed in the Morgue Specialization Department of Presidency of Forensic Medicine Institution between 2008 and 2014 were retrospectively evaluated. In 34 cases, the cause of death was found to be choking; 61.7% of the cases were male and 38.3% were female. The mean age was 34.9 years. Choking material was detected in all cases except one. The most common choking material was termed as "food," while the kind of food could not be determined as examination revealed mixtures of more than one food. Clinical history was described based on eye-witness reports and medical history was available only in 11 (32.3%) cases. The obstructing choking material was removed during autopsy in 30 (88.2%) cases and resuscitation in 4 (11.8%) cases. Toxicological examination revealed no substance in 15 (44.1%) cases, while drugs used in psychiatric diseases (antipsychotic, antiepileptic, antidepressant, etc.) and ethanol were detected in nine (26,5%) and six (17.6%) cases, respectively.

In conclusion, predisposing factors that play a role in the development of choking and the location of these factors in the mechanism of death are important. In cases with a suspicion of choking, it is important to take a detailed clinical history, learn if resuscitation has been performed, and determine if choking material has been removed prior to autopsy. The oropharynx, teeth, entire respiratory tract, upper gastrointestinal system, and gastric content must be examined; toxicological analysis is also recommended. Patients with predisposing factors and their relatives should be informed about this life-threatening event and multidisciplinary approaches should be adopted in case of need.

Choking, Café Coronary, Food

H58 A Mercury "Bullet" at Autopsy

Sarah Long, BS, 1352 Smither Drive, Huntsville, TX 77340; Richard Wiggins, BS, American Forensics, 2452 U.S. Highway 80, E, Mesquite, TX 75149; Jennifer T. Akin, MS, American Forensics, PO Box 550846, Dallas, TX 75355; and Amy C. Gruszecki, DO, American Forensics, LLC, PO Box 550846, Dallas, TX 75355*

After attending this presentation, attendees will understand how to better identify exploding ammunition from regular ammunition, emphasizing the importance of the autopsy examination with adjunct toxicology to determine the manner and cause of death when multiple possible methods for a suicide attempt have been used.

This presentation will impact the forensic science community by demonstrating an autopsy of an individual with unique findings which have not previously been described in scientific literature. This presentation will expand the understanding as well as the identification of exploding ammunition involving mercury as it impacts the human body. This presentation will provide a better appreciation of ammunition identification and toxicology testing as adjunct investigative tools.

Contrary to other methods that result in much lower case fatalities, firearm suicides continue to increase in the United States. Though suicides can be impulsive, the lethality of the method selected can be a critical determinant of whether the attempt is fatal or not.[1] Suicide in which multiple methods are used are uncommon, but not rare, accounting for 1.5%-5% of all suicide cases.[2] Defined as the application of more than one mechanism of death, a complex suicide (either planned or unplanned) ensures a fatal outcome.[2] The possibility of exploding handgun ammunition for civilian use necessitates an understanding of the diagnostic features as well as the characteristics of such ammunition. As the majority of suicide cases result from firearms, forensic pathologists should be cognizant of exploding bullets and consider their effects in future cases.

In this case study, a 62-year-old man initially appeared to have committed suicide via a gunshot wound to the abdomen. At time of autopsy, postmortem radiographs were taken to document the location of the projectile. Despite only one gunshot wound present on the external examination of the body, two apparent projectiles appeared on the radiograph. After the internal examination, what was thought to be the second bullet was in fact a spherical glob of liquid mercury. Closer examination of the radiograph showed minute pellets of mercury throughout the large and small intestine. In order to determine if the mercury had been ingested or was part of a mercury-altered exploding projectile, blood was tested for mercury levels. Blood levels of mercury were 33mcg/L with the normal range being less than 10mcg/L.

Exploding ammunition when fired projects a small group of missile fragments to the target, but do not result in greater tissue damage.[3] Thus, exploding ammunition is anatomically indistinguishable from conventional ammunition. Identification relies on the forensic pathologist's ability to identify both a primer cup and anvil in a radiograph and/or during the internal examination so that the necessary safety precautions can be taken.

Although the cause of death may appear clear prior to autopsy, this case study demonstrates the importance of a complete autopsy and of a forensic pathologist being familiar with the identification and understanding of the various types of ammunition currently available and their effects on the human body. In addition, this case highlights another case of suicide in which at least two methods were attempted.

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Mercury, Bullet, Suicide

H59 Chain Saw-Related Fatalities: What Is All the Buzz About?

Abigail J. Grande, BS, WMU Homer Stryker MD School of Medicine, 1000 Oakland Drive, Kalamazoo, MI 49008; Shawn A. Silver, BS, 4648 Wendrick Drive, West Bloomfield, MI 48323; Joseph A. Prahlow, MD, Western Michigan University School of Medicine, 300 Portage Street, Kalamazoo, MI 49007; and Joyce L. deJong, DO, WMU Homer Stryker MD, School of Medicine, Dept of Pathology, 1000 Oakland Drive, Kalamazoo, MI 49008*

After attending this presentation, attendees will possess a more detailed understanding of the settings and subsequent etiology of chain saw-related accidents and better appreciate the associated risk factors.

This presentation will impact the forensic science community by increasing the confidence of the medical examiner and death investigation team while analyzing the circumstances of this rare, yet challenging, situation. Attendees will be able to further utilize the information presented to promote safer practices while handling chain saws.

According to the Occupational Safety and Health Administration (OSHA), there are approximately 28,000 chain saw-related injuries annually and in 2012, OSHA reported 243 work-related deaths involving tree trimming and clearing activities.[1] Furthermore, accidental, non-work-related fatalities during use of a chain saw remains a public health concern for many areas of the country.

This study examined a series of 14 cases from 2008 to 2013 in which death was associated with chain saw use. Ten of these cases were classified as accidents and the remaining three as natural. There were no cases of homicide or suicide. There were 12 males and 2 females with the mean age of the decedent being 49 years. Although fatalities while using a chain saw are rare, similar patterns of circumstance can provide medical examiners and death investigators with tools to better classify the etiology of the accident.

Accidents involving chain saws can be grouped based on decedent as either: (1) saw operator; or, (2) bystander. Of the saw operator category, one can further classify the accidents into three categories: (1) kickback-related injuries; (2) falling tree limbs; and, (3) fall from a height. Kickback-related injuries occur when the rotating chain is stopped suddenly by contact with a more solid area, throwing the saw rapidly backward toward the operator.[2] Cases presented will highlight the various mechanisms in which falling tree limbs as well as falls from elevation during chain saw operation can lead to fatality. The two accidents classified to the bystander category will be presented to highlight the importance of operator awareness and safety with regard to falling tree limbs. Through the above selected cases, and literature review of common injury patterns, medical examiners will have an increased level of awareness of the likely distribution and pattern of injuries associated with each of the individual categories.

The examination of natural deaths surrounding the use of chain saws followed a somewhat consistent pattern as well. Although the sample size was small (n=3), each individual had a significant history of cardiovascular disease and/or coronary artery disease. Each individual was witnessed using a chainsaw for yard work and then, after a period of time ranging from minutes to a few hours, was found deceased either within the home or in the yard.

Chain saw production companies, the logging and forestry industry, and public safety agencies go to great lengths to provide safety information to their users; however, when dealing with elements of nature and occasionally unpredictable situations, it is improbable to safeguard against everything. By better understanding the etiology of accidental chain saw-related deaths, the forensic science community will not only be more equipped to promote and improve safety techniques but will also have an increased level of confidence during death investigation in this challenging situation.

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Chain Saw Death, Accident, Injury

H60 Intrepretation of Pedestrian Injuries: A Collaborative Research Approach

Jeffrey M. Jentzen, MD, University of Michigan, 300 N Ingalls, NI2D19 - SPC 5452, Ann Arbor, MI 48109; Joel B. MacWilliams, BA, University of Michigan ICAM, 1150 W Medical Center Drive, 3328 Med Sci, Ann Arbor, MI 48109-5677; Diana I. French, BA, University of Michigan-Pathology, NI2D19 NIB, 300 N Ingalls, Ann Arbor, MI 48109-5452; and Stewart C. Wang, MD, University of Michigan ICAM, 1150 W Medical Center Drive, 3328 Med Sci I, Ann Arbor, MI 48109-5677*

After attending this presentation, attendees will be better prepared to identify and document injury and trauma specific to pedestrian/vehicle collisions. Attendees will gain a better understanding of the mechanisms of pedestrian trauma and the anatomic morphomics related to pedestrian trauma.

This presentation will impact the forensic science community by equipping death investigators and forensic pathologists with the tools to identify the unique and subtle injuries associated with pedestrian injuries.

Despite improvements in decreasing automobile injury and deaths, pedestrian/motor vehicle deaths and injuries continue to rise at significant levels, causing an international crisis. In 2013, 4,735 pedestrian deaths and 69,000 injuries were reported in the United States; 8,000 and 300,000 in the European Union; 3,300 and 27,000 in Japan; and 3,600 and 90,000 in Korea, respectively. The National Transportation Safety Board (NTSB) along with major car manufacturers have made an increasing effort to understand the nature of pedestrian crashes and injuries in an attempt to provide saver pedestrian environment and vehicles. The United Nations recently has created a Global Technology Regulation No. 9 to reduce the level of injury sustained by pedestrians in frontal impacts.

The International Center for Automotive Medicine (ICAM), located on the campus of the University of Michigan Hospitals, was one of the founding members of the Crash Injury Research Engineering Network (CIREN) sponsored by National Highway Traffic Safety Administration (NHTSA). ICAM's trifold mission is to foster synergistic *research* between medical specialties, medical treatment and cross-disciplinary *education*, and *policy formulation* regarding federal rules and testing for the automotive industry. ICAM utilizes "analytical morphomics" through the processing of 3D medical images to gain deeper understanding into the cause of injuries so they can be better prevented and treated. This presentation will discuss the ongoing research program into pedestrian/motor vehicular fatalities in Southeastern Michigan between ICAM and the Washtenaw County Medical Examiner Office.

The frequent sites of pedestrian injuries include the adult and child head followed by adult leg regions. Sixty-five percent of fatal injuries occur at speeds less than 40 miles per hour. The highest frequency of contact between the pedestrian victim and the vehicle includes the bumper, grill/hood edge, hood and top fenders, and windshield including the A-pillars. Pedestrian injury data is severely outdated and of limited quantity.

ICAM solicited a number of partners in Southeastern Michigan including medical examiners, trauma centers, Michigan Department of Transportation, and various law enforcement agencies. Cases were selected using the following criteria: pedestrians in upright positions; frontal vehicular impact; no add-on equipment (snowblades, etc.); vehicles must be MY2000 or newer; and limited impacts of less than 40mph. Law enforcement and medical examiner departments contact the senior crash investigator to evaluate the scene of death and schedule a full-body Computed Tomography (CT) scan. A complete autopsy is performed with the prosector initially blinded to the results of the radiology examination. Data analysis includes primary sources of vehicle and road data, exemplar vehicle inspections, medical data and autopsy reports, injury lists, and mapping and CT scan data. The results of the CT and autopsy studies were correlated and developed for the creation of injury lists and overall formal case review. To date, approximately 15 cases have been evaluated.

Preliminary findings confirm the discrepancies between CT skeletal injuries and soft tissue injuries identified at autopsy. CT and autopsy studies are complementary and provide an overall view of the injury patterns encountered in pedestrian collisions. Fractures of transverse vertebral processes and scapula are commonly found in pedestrian injuries. The evaluation of anatomical morphomics such as psoas muscle cross section, Body Mass Index (BMI), rib characteristics, and others provides prediction models for survival. It is anticipated that upon completion this study will provide essential impact/injury and morphomic data that will revolutionize automobile design and improve pedestrian safety.

Pedestrian Injuries, Motor Vehicle Collisions, Analytical Morphomics

H61 Characteristics of Traffic Crash-Related Blunt Traumatic Aortic Injury (BTAI)

Michael Freeman, MD, PhD, Oregon Health & Science University School of Med, 425 NW 10th Avenue, Ste 306, Portland, OR 97209; Todd M. Luckasevic, DO, Alleghey County ME, 1520 Penn Avenue, Pittsburgh, PA 15222; Karl E. Williams, MD, Allegheny County OME, 1520 Penn Avenue, Pittsburgh, PA 15222; and Anders Eriksson, MD, PhD, Umea University, Dept Forensic Medicine, PO Box 7616, Umea SE-907 12, SWEDEN*

The goal of this presentation is to provide an overview of crash-related BTAIs, the characteristics of the occupants who sustain them, the crashes in which they occur, and the survival outcomes for those victims.

This presentation will impact the forensic science community by describing the distribution and characteristics of a relatively common cause of death in traffic accidents.

Traumatic rupture of the aorta is associated with a high risk of death and results from high-energy blunt trauma of the chest. Prior studies have estimated that traffic crashes account for 50% to 90% of BTAI with injury found on autopsy in one in three crash-related deaths.[1,2] The most common location of aortic rupture, typically a transection, is at the aortic isthmus, near the location of the ligamentum arteriosum. The injury mechanism is related to the fact that the descending thoracic aorta is fixed to the posterior chest wall while the heart and great vessels are relatively mobile, resulting in shear and tension forces at the site of the rupture.

While the death rate from BTAI has been reported to be as high as 90%, the actual rate is difficult to judge because of a lack of large studies and the fact that study populations tend to be biased toward survival (hospital cohorts) or death (autopsy studies).

To address this gap in knowledge, this study queried the National Automotive Sampling System-Crashworthiness Data System (NASS-CDS) of the National Highway Traffic Safety Administration for crash-related BTAI occurring from 1993 through 2011. The NASS-CDS investigates approximately 5,000 crashes every year in 36 geographic Primary Sampling Units (PSU). The data are weighted to provide a national estimate of all police-reported crashes and associated injuries occurring in the United States and involving passenger vehicles.

The results of the analysis were as follows: There were an estimated 61,209 cases of BTAI during the 19-year period, an average of 3,222 cases per year, with the number of cases decreasing over the study period (see Figure 1). There were 39,170 of the cases that were reported as fatalities (64%) and 22,039 (36%) reported as non-fatal (Figure 2). The majority were transections (52%), followed by major ruptures with hemorrhage outside the mediastinum (31%) and confined to the mediastinum (16%).

The average age among the fatalities and non-fatal cases was 40.5 years and 33.2 years, respectively. Men comprised 58.5% of the group and women 41.5%. There was no gender disparity in the fatality rate.

Among all of the BTAI cases, front and side impact crashes accounted for half of the collision types (23.7% and 23.4%, respectively). Ejections were relatively common in the population: 10.6% were completely ejected and another 6.7% were partially ejected. Even more common was the failure to use a seatbelt: 65.5% of BTAI occurred in unrestrained occupants. Drivers comprised 56.7% of the occupants and 26.6% were front seat passengers.

Injuries were most commonly attributed to impact with the steering wheel (22.4% of all cases, accounting for half of all driver's injuries), followed by right interior (21.1% of all cases and approximately 80% of all front passenger injuries).

For the crashes that could be reconstructed for speed change (the non-rollovers primarily), the average delta V of the collision was 32.3mph (51.7 km/h). The relationship between delta V and fatality risk can be seen in the binomial logistic regression represented in Figure 3. Vehicle-to-vehicle impacts comprised 74.0% of the collisions and 20.1% were impacts with narrow objects such as trees or poles.

The present investigation represents the first analysis of BTAI based on national crash data and provides new insight into the nature of these injuries.

Figure 1. Estimated annual number of crash-related BTAI cases in the United States 1994-2011

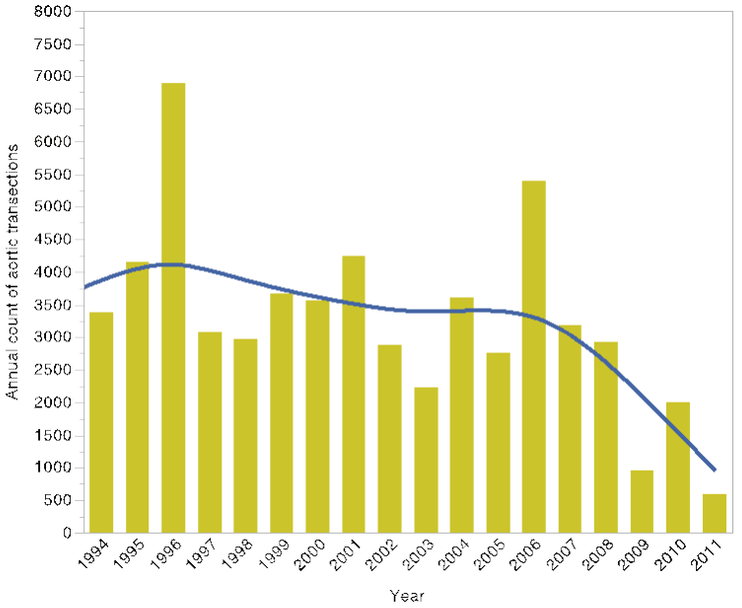
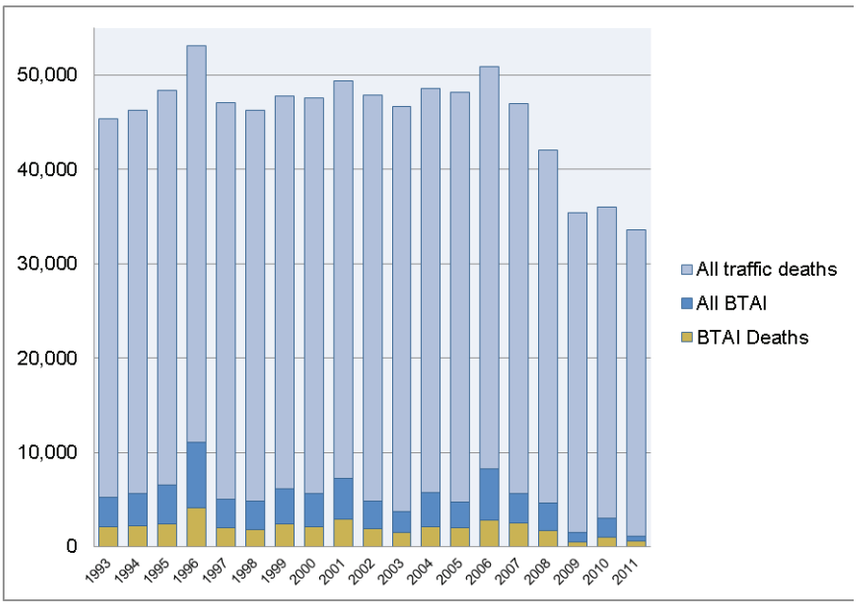
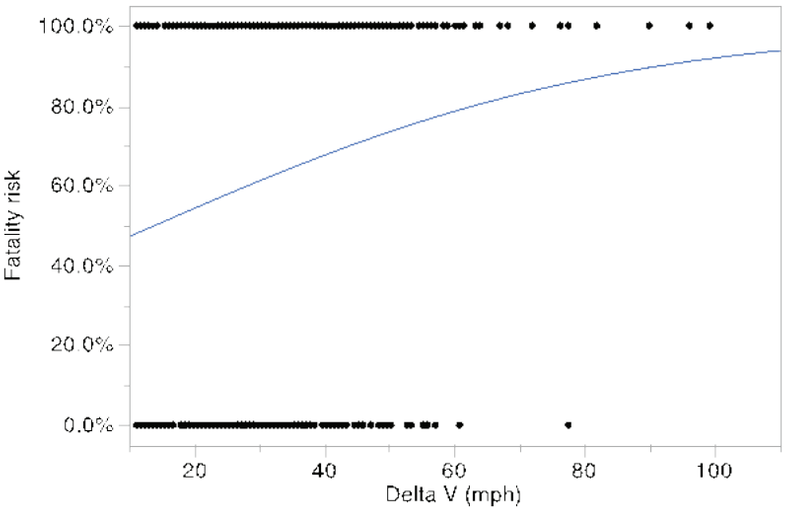


Figure 2. Estimated annual number of all crash-related BTAI and BTAI fatalities versus the total number of crash related deaths



*Presenting Author

Figure 3. Risk of fatal versus non-fatal BTAI as a function of crash severity (delta V in mph)



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Blunt Traumatic Aortic Injury, Rupture, Transection

H62 If at First You Don't Succeed…

Richard C. Fries, DO, 3741 Modlin Avenue, #2, Fort Worth, TX 76107; Tasha Z. Greenberg, MD, Tarrant County MEO, 200 Feliks Gwozdz Place, Fort Worth, TX 76104-4919; Nizam Peerwani, MD, Tarrant County OCME, 200 Feliks Gwozdz Place, Fort Worth, TX 76104-4919; and Allison Mautone, MD, 800 Cotton Depot Lane, #128, Fort Worth, TX*

After attending this presentation, attendees will be familiar with suicide cases by multiple gunshot wounds and the characteristics that distinguish these suicides from homicide cases.

This presentation will impact the forensic science community by illustrating patterns observed in multiple gunshot wound suicides at the Tarrant County Medical Examiner's Office to assist with the evaluation of similar cases that are inherently suspicious for personnel involved.

Although there are several reports of suicide by firearm with more than a single wound path, these cases are inherently suspicious for everyone involved. A ruling of suicide as the manner of death is frequently met with resistance by law enforcement and especially family members. Misconceptions about the possibility, frequency, and lack of understanding of anatomy are major contributors to objections of a suicide ruling with multiple gunshot wounds. These factors may cause the manner of death to be undetermined, which may cause an underestimation of the incidence of cases of suicide by multiple gunshot wounds.

A retrospective analysis of suicides with multiple gunshot wounds over the past 12 years identified 20 cases. For the time frame analyzed, the overall firearm suicide rate was 49% with multiple gunshot suicides comprising 1.2% of this total. The firearm suicides were 84% men and 88% White; a breakdown of the demographic information on the multiple gunshot suicides determined the cases predominantly involved middle-aged White men. Scene investigation data reveals that these cases predominantly occurred at home or on the surrounding property and in less than half of the cases was a note discovered at the scene or any verbal admission of a self-inflicted wound made. Approximately half of the cases had a history of depression based on reported history or toxicology testing positive for antidepressant medication; previous suicide attempts were reported in none of the cases. Almost all of the firearms used were large caliber, with a small subset of cases involving the use of more than one firearm. The cases were predominantly composed of two gunshot wounds, although up to four gunshot wounds were observed. The vast majority of total entrance wounds were contact with few exceptions due to specific case circumstances. In terms of body region, the head was the most common target as both the only target and in combination with other body regions. The chest was the next most common target, with only a small number of cases involving the abdomen. Over the time frame analyzed, four years had no cases of multiple gunshot wound suicides identified and the years with identified cases all had similar numbers.

Suicide, Firearm, Multiple Gunshot Wounds

H63 Pathologist Consensus in the Interpretation of Patterned Injuries From Photographs: Reasons for Lack of Consensus

William R. Oliver, MD, East Carolina University, Brody School of Medicine, Brody Medical Sciences Bldg, Greenville, NC 27834*

After attending this presentation, attendees will have a greater understanding of the cognitive basis for disagreements in pathologist interpretation of patterned injuries of the skin from photographs.

This presentation will impact the forensic science community by providing an explanation of the cognitive and practical issues surrounding the diagnosis of patterned injuries of the skin. In particular, this presentation will address real versus apparent discordance in interpretation.

This presentation represents the second of three surveys supported by the National Institute of Justice examining how forensic pathologists interpret photographs of patterned injuries of the skin. The project was originally proposed as a method of studying the effects of image processing on the diagnoses of patterned injuries of the skin from photographs. It was to consist of three surveys. The first survey was to be of "classic" patterned injuries to establish a baseline of high confidence and consensus. The second survey was to be of degraded images to study the effects of degradation on that consensus. The third survey was to add image processing, specifically contrast enhancement, to the images and see if this affected diagnosis in the same way similar studies did for radiology diagnosis.

However, the results of the first study were a complete surprise. Rather than a consistent high consensus and confidence, there was a wide variation of both, with consensus ranging from a high in the mid-90% to a low in the upper-20%. This was counter to the assumption that all but a few images should have been trivial to diagnose for all pathologists.

The principal findings of the first survey were presented at the 2015 American Academy of Forensic Sciences Annual Scientific Meeting.[1] The diagnoses were requested in "tiers" going from general (e.g., "blunt vs. sharp injury") to moderate (e.g., "abrasion vs. contusion") to specific (e.g., "baton vs. hammer"). The hypothesis was that there would be more consensus at the more general tiers and less consensus at the more specific. This was not the case — there was lower consensus at the middle tier and no significant difference between the most specific and most general. A second finding was that there was an extraordinarily high correlation between confidence and consensus when all pathologists' answers were considered in aggregate, but the correlation was low at the individual pathologist level. A number of demographic features correlated with choosing the consensus diagnosis, with the most important predictor being actively performing autopsies.

Modification of the second survey occurred to investigate these intriguing findings rather than look at degraded images. Those who had responded to the first survey were asked to review their answers and indicate why they had different responses or low certainty. These results are still being analyzed but the initial findings are also fascinating. The most common reasons for lack of consensus were: (1) that the injuries were not specific and required history for determination; (2) differences in nomenclature (e.g., "I meant the same thing, but I use a different term"); (3) the presence of multiple injuries; and, (4) difficulty in taking the exam (e.g., "I pushed the wrong button" — most common with the confidence slider). While image quality was asked about and played a part, it was a minor issue.

The problem of multiple injuries is most common with blunt trauma. It is the case that with blunt trauma, injuries are rarely of one type — there are bruises and abrasions, or abraded lacerations, etc. The question regarding which injury was the "primary" or "dominant" injury was asked, but many of the respondents simply refused to make that distinction.

Of greater interest was the reliance on history. In the most specific tier of questions, development of the questions occurred so as to have one clearly obvious true result and others that were (potentially) much more unlikely (though not necessarily impossible), with participants being asked which was most likely. The respondents often commented that there were possibilities that were not in the list and the only way to tell the difference was with history or other information. Some commenters noted that they had been trained to decline to make diagnoses in the absence of history and felt that making such a diagnosis given just a photograph was malpractice.

The statistics of these findings will be presented as well as a discussion of the sources of disagreement.

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Forensic Image Analysis, Patterned Injuries of the Skin, Forensic Pathology Diagnosis

H64 Effect of Angled Impact on Bone Fracture Pattern

Jacob E. Hoerter, Bellarmine University, 2001 Newburg Road, Louisville, KY 40205; David J. Porta, PhD, Bellarmine University, Dept of Biology, 2001 Newburg Road, Louisville, KY 40205; and Tyler A. Kress, PhD, BEST Engineering, 2312 Craig Cove Road, Knoxville, TN*

After attending this presentation, attendees will gain a deeper understanding of the different fracture patterns that may result from angled versus perpendicular impacts to human long bones.

This presentation will impact the forensic science community by providing information to better analyze fracture patterns and determine more precise mechanisms of injury or rule out potential causes of fractures.

The complex protein structure and mineral composition of bone provide phenomenal tensile strength despite being low in density. The unique structure of bone yields a strange array of fracture patterns that depend on the mechanism of force, be it torsion, compression, or impact. Fracture patterns provide valuable insight into the nature of the applied force that resulted in the injury, particularly if the mechanism of fracture was not observed. In the field of forensic science, there is an obvious advantage to understanding fracture mechanics when testifying about the cause of a particular injury. Though previous studies have examined patterns that result from changes in impact velocity and force, few other factors have been investigated, such as the specific angle of an impact and how it might affect fracture pattern. Some have claimed that particular fracture patterns (e.g., transverse versus oblique) can be attributed to a specific angle of impact. It was previously shown in this study's laboratory that perpendicular impacts result in typical bending-type fractures that include transverse, oblique, and wedge (a.k.a. butterfly) patterns.

For this project, 21 human long bones (five humeri, eight femurs, and eight tibias) were subjected to angled impacts in order to study the resultant patterns. The bones were retrieved from five embalmed cadaver donors. Two died from cancer (76yF and 46yM), two from coronary disease (91yF and 90yF), and one from a stroke (84yM). Each bone was impacted approximately mid-shaft on its anterior surface with a 4.75cm-diameter hardened steel pipe in a custom-built spring-loaded impact machine. Impact force was recorded using a load cell and the signal was fed to a personal computer. Data were formed into plots of time versus force via specialized software. Left-side long bones were impacted at a 60-65 degree angle with respect to the long axis of the bone. Their right-side counterparts were impacted at 70-75 degrees. Each trial was recorded on high-speed video (300fps) and resultant fractures were documented photographically.

All three classical fracture patterns (oblique, transverse, and wedge) as well as comminuted fractures were observed as a result of the angled impacts. Of the 21 fractures, 38% were oblique, 24% transverse, 24% comminuted, and 14% wedge. The perpendicular impacts of 109 bones (tibias and femurs) previously observed in this study's laboratory resulted in 26% oblique, 15% transverse, 34% comminuted, and 26% wedge.

The results of this study indicate that impact or bending fractures result in a variety of patterns regardless of impact angle. Fracture pattern, therefore, cannot be used to determine angle of impact. Moving forward, force data from these fractures will be correlated with mineral composition and calcium content (by bone ashing and calcium atomic absorption spectroscopy) to investigate their potential role in fracture patterns.

Angle, Impact, Fracture

H65 Sudden Unexpected Deaths Due to Sarcoidosis: A Forensic Autopsy Study

Tiantong Yang, Haidian District, 26 Houtun South Road, Beijing 100192, CHINA; Xiang Zhang, MD*, OCME, 900 W Baltimore Street, Baltimore, MD 21223; Zhaoming Guo, MD*, Institute of Evidence Law and Forensic Science, NO.26, Houtun South Road, Haidian District, Beijing 100192, CHINA; Allen Burke, MD, University of Maryland School of Medicine, 22 S Greene Street, NBW51, Baltimore, MD 21201; Mary G. Ripple, MD, 900 W Baltimore Street, Baltimore, MD 21223; David R. Fowler, MD, OCME, 900 W Baltimore Street, Baltimore, MD 21223; and Ling Li, MD, OCME, State of Maryland, 900 W Baltimore Street, Baltimore, MD 21223*

After attending this presentation, attendees will better understand sudden death due to sarcoidosis, particularly the pathological distribution and pattern of sarcoidosis. The clinicopathological correlation of sarcoidosis deaths will also be addressed.

This presentation will impact the forensic science community by providing attendees with a deeper understanding of the role forensic pathologists/medical examiners play in the determination of the cause of sudden death. This presentation will also demonstrate the inter-relationship between forensic pathologists and clinicians in counseling family members about sarcoidosis.

Sarcoidosis is a multisystem disease of uncertain etiology characterized by multifocal areas of discrete and confluent granulomatous inflammation that may be responsible for sudden and unexpected death.[1,2] Sarcoidosis affects young and middle-aged adults without sex predilection.[1] Environmental, occupational, and infectious causes may act as immunologic triggers in genetically predisposed individuals.[2] Sarcoidosis commonly involves the lymph nodes, lungs, cardiovascular system, liver, spleen, central nervous system, and kidney. Involvement can be widespread or limited to involvement of only a single system at a time. The failure to diagnose sarcoidosis clinically is partly attributable to the relative rarity of clinically apparent forms of the disease.[3,4] In a significant proportion of patients with sarcoidosis, the initial presentation is sudden death.[3] There have been few reported autopsy series of patterns of multisystem involvement by sarcoidosis.

A retrospective search of deaths caused by sarcoidosis was performed from the Office of the Chief Medical Examiner, State of Maryland, over a seven-year period from 2005 to 2011. In all cases, medical history, circumstance of death, and autopsy findings including toxicological testing results were reviewed. Distribution of disease was determined both by gross and microscopic examination. Gross evaluation included heart weight and measurements of the ventricular septum and left ventricular free wall thicknesses. Histologic sections were taken in a standardized way and included the Lateral Left Ventricular (LLV) wall, Posterior Left Ventricle (PLV), Anterior Left Ventricle (ALV), right ventricular free wall, interventricular septum, and interatrial septum. Cardiomegaly was defined based on established criteria depending on body weight.4.

A total of 6,442 natural deaths were identified and sarcoidosis was listed as cause of death in 29 cases (0.62%). Of 29 sarcoidosis cases, cause of death was certified as cardiac arrhythmia due to sarcoidosis in 25 cases and pulmonary sarcoidosis in four cases. Of the 25 cardiac sarcoidosis deaths, sarcoid lesions involved left ventricle in 24 cases, followed by interventricular septum (N=20), right ventricle (N=11), mitral and tricuspid valve (N=7), and interatrial septum (N=2). Other organ system involvement included lung in 20 cases, followed by lymph nodes (N=17), liver (N=7), spleen (N=6), and brain (N=1). Of the four pulmonary sarcoidosis cases, two had extensive granulomatous change with diffuse pulmonary fibrosis, one complicated by bronchopneumonia, and one complicated by hypothermia and cocaine use. Other organ system involvement included lymph node (N=4), spleen (N=2), liver (N=1), kidney (N=1), and brain (N=1). Of the 29 cases, 11 patients were witnessed sudden collapse and 18 were unwitnessed. Twenty-one (21/29) cases were clinically undiagnosed. The majority (76%, 19/25) of the cardiac sarcoidosis cases were either overweight (N=4) or obese (N=15), whereas, the body mass index was normal in all four pulmonary sarcoidosis cases.

In conclusion, presented here are characteristics of a series of autopsy cases of sarcoidosis from a state-wide single medical examiner's office with review of literature and clinicopathological correlation.

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4. Kitzman D.W., Scholz D.G., Hagen P.T., Ilstrup D.M., Edwards W.D. Age-related changes in normal human hearts during the first 10 decades of life. Part ii (maturity): A quantitative anatomic study of 765 specimens from subjects 20 to 99 years old. *Mayo Clin Proc*. 1988; 63:137-146

Sarcoidosis, Non-Caseating Granulomas, Sudden Death

H66 Sudden Unexpected Deaths Due to Intracranial Meningioma: A Presentation of Six Fatal Cases and a Discussion of the Mechanisms of Death

Lorenzo Gitto, MD, Sapienza University, Dept of Anatomy, Histology, Forensic Medicine, & Orthopedics, Viale Regina Elena, 336, Rome 00169, ITALY; Stephen J. Cina, MD, 505 N Lake Shore Drive, Unit 2701, Chicago, IL 60611; James A. Filkins, MD, JD, PhD, PO Box 6237, Villa Park, IL 60181; and Serenella Serinelli, MD, Sapienza University, Dept of Anatomy, Histology, Forensic Medicine, & Orthopedics, Viale Regina Elena 336, Rome, Lazio 00169, ITALY*

After attending this presentation, attendees will have a better understanding of the importance of sudden death due to undiagnosed meningiomas in subjects in apparently good health found dead in the absence of known causes.

This presentation will impact the forensic science community by highlighting the pathological theories that explain sudden deaths due to meningioma.

Intracranial tumors usually produce clinical signs and symptoms due to a combination of local tissue compression and damage, edema, and the alteration and shift of intracranial structures. An individual may present with focal neurological signs and symptoms as a result of the particular location of the neoplasm or with generalized symptoms due to increased intracranial pressure that may include headache, nausea and vomiting, altered mental status, papilledema, and seizures. The severity of these clinical manifestations is often progressive and directly related to tumor growth. Death usually occurs after a variable period of time characterized by a declining clinical course. Occasionally, sudden death due to asymptomatic intracranial neoplasms may be encountered in forensic settings.

Meningiomas arise from the dura mater and are composed of neoplastic meningothelial (arachnoidal cap) cells. Many meningiomas are found incidentally following neuroimaging for unrelated reasons. Family history studies suggest a role for inherited susceptibility for meningioma in addition to multiple genetic abnormalities involving different genes.

Deaths due to meningiomas are routinely diagnosed in clinical practice because this neoplasm tends to present with the typical progression of neurological deficits. On the other hand, sudden unexpected death due to meningiomas are rarely described in the literature. Six fatal cases of previously undiagnosed intracranial meningiomas from the Cook County Medical Examiner's Office from 1998 to 2014 are presented.

Case 1: Meningioma of the cerebellopontine angle.

Case 2: Meningioma of the middle cerebral fossa in the area of the left temporal lobe.

Case 3: Suprasellar meningioma.

Case 4: Meningioma of the basilar portion of the occipital bone.

Case 5: Meningioma (based on clinical history — not specified).

Case 6: Intraventricular meningioma.

The most common explanation of the mechanism of sudden death due to intracranial neoplasms — despite their vast biological and morphological diversity — is a rapid increase in intracranial pressure produced by the mass effect of the neoplasm. Increased intracranial pressure may in turn lead to seizures, acute hydrocephalous, brain edema, hypothalamic dysfunction, herniation due to mass effect, and brainstem compression with death due to direct involvement of respiratory and cardiac centers. Other mechanisms of death include acute intracranial and intra-tumoral hemorrhage and benign neoplasms that grow in the vicinity of vital centers (such as the hypothalamus) disrupting thermoregulation or neural discharge in autonomic pathways leading to cardiac suppression or lethal arrhythmias.

Forensic pathologists must keep in mind that sudden unexpected death caused by intracranial meningiomas, although extremely rare, may be encountered in the forensic setting. In cases of sudden death due to intracranial meningiomas or other intracranial neoplasms, consultation with a neuropathologist may be useful. Accurate diagnosis of the tumor may assist family members of the deceased by identifying any genetic or environmental risk factors and benefit public health by providing information about the natural evolution of untreated disease.

Sudden Death, Meningioma, Mechanisms of Death

H67 Non-Rheumatoid Fibrinous Pericarditis: A Medical Examiner Quest With an Update on Myocarditis and Use of Molecular Diagnostic Techniques

Avneesh Gupta, MD, University of Michigan/Wayne County MEO, 1300 E Warren Avenue, Detroit, MI 48207; Kilak Kesha, MD, Wayne County MEO, 1300 E Warren Avenue, Detroit, MI 488207; Francisco J. Diaz, MD, 1300 E Warren Avenue, Detroit, MI;and Carl J. Schmidt, MD, Wayne County MEO, 1300 Warren, Detroit, MI 48207*

After attending this presentation, attendees will have a better understanding of non-rheumatoid fibrinous pericarditis etiology, pathogenesis, and autopsy presentations.

This presentation will impact the forensic science community by highlighting the use of new molecular modalities and how these modalities apply to medical examiners.

The classic "bread and butter" appearance of fibrinous pericarditis has been described in rheumatic disease. Other infrequent causes vary from other immunological diseases such as Systemic Lupus Erythematosus (SLE), post myocardial infarct, uremia, tuberculosis, radiation effects, and bacterial and viral etiology. In most of the described cases, pericarditis occurs as a delayed complication.

Presented is a case of a 21-year-old White female who was seen in the emergency room to rule out pulmonary embolism for shortness of breath, chest pain, and light headedness. Pulmonary embolism was ruled out by negative findings at Computed Tomography (CT) scan and Ventilation/Perfusion (V/Q) scan. Her laboratory tests showed increased counts of acute inflammatory cells in her blood. Two days after her initial presentation, she was examined for lower back pain in an urgent care setting and released home after a negative cardiac work up. She died at home and the case was investigated by the medical examiner's office as a sudden death. Autopsy showed collection of serous fluid into the pericardial sac with a bread and butter appearance. Microscopically, pericardium showed acute inflammation with fibrinous exudates. Sections of heart showed areas of lymphocytic infiltration with acute fibrinous inflammation of the pericardium. Vasculitis was seen in the small heart blood vessels and was negative in other organs. No granuloma or necrotizing lesion was seen in microscopic sections of all organs, including the heart, ruling out a rheumatic arthritis.

The present study highlights the quest for non-rheumatic disorder as cause of pericarditis. Other uncommon common etiological entities were considered based on review of medical charts and clinical presentation. Special stains for bacterial and fungal elements performed on heart tissue were unremarkable. As foci of lymphocytic infiltration had more than 14 lymphocytes per mm^2 with myocyte necrosis; represented histological diagnostic for myocarditis (viral) based on Dallas criteria.[1] Molecular studies were performed on heart tissue blocks for identification of cardiotropic viruses. Human Parvo Virus B 19 (PVB19) was isolated from heart tissue blocks. In European studies, PVB19 was mainly detected in patients with biopsy-proven myocarditis.[2,3] The present case study highlights updates on pathophysiology, a diagnostic criteria for myocarditis along with the use of new molecular techniques for the detection of idiopathic cardiomyopathies in a medical examiner setup.

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Fibrinous Pericarditis, Molecular Diagnosis, Myocarditis

H68 Liver Pathology at Autopsy in First Presentation of Diabetic Ketoacidosis (DKA)

Anita Lal, MD, 25 Morton Shulman Avenue, Toronto, ON M3M 0B1, CANADA; Jacqueline L. Parai, MD, Ottawa Hospital, Division of Anatomical Pathology, 501 Smyth Road, Box 117, 4th Fl, Ottawa, ON K1H 8L6, CANADA; and Chris Milroy, MD, LLB, Ottawa Hospital, 501 Smyth Road, Box 117, 4th Fl CCW, Ottawa, ON K1H 8L6, CANADA*

After attending this presentation, attendees will understand the liver pathology seen on first presentation of DKA.

This presentation will impact the forensic science community by indicating the frequency of deaths during first presentation of diabetes mellitus and the pattern of liver pathology in these cases.

Ketoacidosis is a common finding at autopsy, representing around 1% of autopsy diagnoses in this study's practice. The two most common reasons for ketoacidosis at autopsy are Diabetic Ketoacidosis (DKA) and Alcoholic Ketoacidosis (AKA).

Liver disease is common in both populations. Deaths from DKA and AKA have previously been analyzed to compare the liver pathology present at death. This study details examination of deaths from diabetic ketoacidosis in which people died on first presentation and were not previously known to be diabetic. Deaths from Toronto and Ottawa, Canada, were studied over a five-year period from 2008 to 2013. The demographics and liver histopathology were examined. There were 21 deaths (13 male, 8 female) identified dying on first presentation of diabetes mellitus out of a total 79 people dying from diabetic ketoacidosis. This represents 26.6% of deaths from DKA. The median age of the first presentation cases was 51 years, mean 51 years, and range 30-68 years. Their Body Mass Index (BMI) ranged from 16 to 40.4, with a median of 25.2 and a mean of 26.3.

Liver histology was available in each case. Each liver was scored according to the method of Kleiner et al.[1] The following were scored: degree (0-3) and location of steatosis (0-3); fibrosis (0-4, with 4 being cirrhosis); portal (0-1) and lobular inflammation (0-2); and presence of glycogenated nuclei (0-1). Two cases could not be scored for steatosis because of decompositional changes. Four cases were not assessed for fibrosis. Four cases could not be assessed for inflammation and three cases for glyogenated nuclei. The findings identified steatosis being present in 18 of 19 cases assessed and was grade 2/3 for degree in 10/19 cases and grade 2/3 for location in 17/19 cases. Fibrosis was grade 2/3 in 10/17 cases. None of the cases was cirrhotic. Inflammation was present in 11/17 cases and glycogenated nuclei were seen in 7/18 cases.

Death during the first presentation of diabetic ketoacidosis forms a significant proportion of ketoacidosis deaths. Non-alcoholic fatty liver disease is present in a high proportion of these patients dying on first presentation of diabetes mellitus, with significant degrees of fibrosis already established. These patients were generally not obese, with a median BMI of 25.2. In view of these findings, the presence of fatty liver disease with fibrosis cannot be assumed to be related to excess alcohol consumption.

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1. Kleiner D.E., Brunt E.M., Van Natta M., et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005:41(6):1313-21.

Diabetic Ketoacidosis, First Presentation, Liver Pathology

H69 DNA Testing in Homicide Investigations

Joseph A. Prahlow, MD, Western Michigan University School of Medicine, 300 Portage Street, Kalamazoo, MI 49007; Thomas J. Cameron, South Bend PD & St. Joseph Co Metro Homicide Unit, 523 E Jefferson Boulevard, South Bend, IN 46617; Alexander Arendt, BS, Mishawaka PD & St. Joseph Co Metro Homicide Unit, 523 E Jefferson Boulevard, South Bend, IN 46617; Kenneth Cornelis, St. Joseph County Metro Homicide, 523 E Jefferson Boulevard, South Bend, IN 46617; Anthony Bontrager, BA, South Bend Police Department, 701 W Sample Street, South Bend, IN 46601; Michael Suth, BS, South Bend Police Department, 701 W Sample Street, South Bend, IN 46601; Lisa B. Black, BS, Indiana State Police Lab, 1550 E 181st Avenue, Lowell, IN 46356; Rebecca Tobey, BS, Indiana State Police, 1550 E 181st Avenue, Lowell, IN 46356; Sharon M. Pollock, BS, 1550 E 181st Avenue, Lowell, IN 46356; Shawn Stur, BS, Indiana State Police, 1550 E 181st Avenue, Lowell, IN 46356; Kenneth Cotter, JD, St. Joseph County Prosecutors Office, County-City Building, South Bend, IN 46601; and Joel Gabrielse, JD, St. Joseph County Probate Court, County Courthouse, South Bend, IN 46601*

After attending this presentation, attendees will: (1) recognize that the value of DNA testing in homicides is not limited to situations of sexual assault; (2) be familiar with a wide range of case types in which DNA testing can play an important role in the investigation and eventual adjudication of purported homicides; (3) understand that a variety of types of evidence may be suitable for DNA testing; and, (4) realize that such evidence may be located at the crime scene, either in the immediate vicinity of the murder victim, or elsewhere — at a sight separate from the main crime scene and/or present on the body or property of the victim or the suspect.

This presentation will impact the forensic science community by focusing attention on the many unique situations in which DNA testing can play an important role in the investigation and eventual prosecution of homicide cases. By examining several unique cases in which DNA testing was instrumental in the investigation, the forensic science community will be better able to recognize situations where similar testing may provide valuable investigative information.

With the advent of forensic DNA testing, and its eventual routine implementation, forensic science has been revolutionized over the past three-plus decades. From the early days when DNA testing first allowed for the "individualization" of persons, the technology has continued to make great progress. DNA testing now allows for the potential definitive identification of perpetrators of sexual assault and other crimes, when suitable evidentiary samples are collected for testing. In addition, DNA testing resulted in numerous exonerations, wherein previously convicted persons have been freed from prison as a direct result of the testing of samples collected and retained prior to the availability of DNA testing.[1,2]

As DNA testing has become even more advanced, the ability to detect trace amounts of DNA has become so incredibly powerful as to produce concerns about potential cross-contamination of evidence by non-perpetrator DNA, whether it originates from the victim, an innocent bystander or other source, or even an investigator or forensic scientist.[3,4] Despite these concerns, the reality is that DNA technology today represents a tool that is so potentially powerful that its usefulness in certain circumstances may not be readily appreciated. In this presentation, a series of homicide cases are presented wherein DNA testing provided valuable investigative information. The cases range from those in which DNA testing was probably not absolutely necessary but provided useful, corroborating information, to those where the DNA test results represented the primary evidence utilized to identify and subsequently charge/try a suspect.

Examples of case types presented include: DNA testing of bloody objects, such as weapons or clothing; DNA testing of a foreign hair found on a homicide victim; evaluation of articles of clothing or other objects for "touch DNA," transferred from simple contact with the skin; DNA testing of a fired bullet at a crime scene; and DNA testing of dog feces at a crime scene.

Cases are presented with emphasis on the unique types of situations where DNA testing may provide valuable investigative information. These cases serve to illustrate the fact that the current state of DNA testing can allow for testing of various items of evidence that, prior to recent advancements in technology, might not have been considered very useful evidence, or if valuable in certain other regards, might not have been considered a viable source for potential DNA testing. All persons involved in homicide investigations, from police to death investigators to forensic pathologists, need to be aware of the powerful capabilities of the current state of DNA testing, and should consider attempting such testing whenever DNA transfer may have occurred in association with a homicide.

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2. Hampikian G., West E., Akselrod O. The genetics of innocence: analysis of 194 U.S. DNA exonerations. *Annu Rev Genomics Hum Genet* 2011;12:97-120.
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5. DNA, Touch DNA, Homicide

H70 Jay Dix Memorial Lecture Series

Michael A. Graham, MD, Saint Louis University School of Medicine, Division of Forensic Pathology, 3556 Caroline, Rm C305, St. Louis, MO 63104; Randy L. Hanzlick, MD, Fulton County Medical Examiner Center, 430 Pryor Street, SW, Atlanta, GA 30312; Joseph A. Prahlow, MD*, Western Michigan University School of Medicine, 300 Portage Street, Kalamazoo, MI 49007; Jonathan Hayes, MD*, OCME, 520 1st Avenue, New York, NY 10016; Keith Pinckard, MD, PhD*, Travis County Medical Examiner (Austin), 1213 Sabine Street, Austin, TX 78701; and Rudy J. Castellani, MD*, University of Maryland, 22 S Greene Street, Baltimore, MD 21201*

After attending this presentation, attendees will better understand how and why deaths related to the topics listed in the lecture series occur. Attendees will learn a systematic approach to the evaluation of such deaths that can easily be implemented in their daily practices.

This presentation will impact the forensic science community by presenting a comprehensive review of what causes and contributes to deaths related to the previously specific topics. Attendees will be able to systematically evaluate deaths in which the previously specified topics may have played a role that they encounter in their daily practices.

Case 1: Electricity is a ubiquitous entity in our daily lives. Some of it is intentionally generated to provide power and some of it originates as a force of nature (lightning). Interaction between humans and electricity is common and typically has no untoward effects; however, under some conditions this interaction may result in morbidity and/or mortality. Multiple causes, mechanisms, and contributory factors play a role in injury and deaths involving electricity. Understanding and evaluating injuries and deaths in which electricity may have played a role requires a basic knowledge of electricity and how it affects various biological vital functions. Recognition of injuries and deaths caused by electricity is particularly important because of implications regarding the safety of others. This lecture will provide a comprehensive review of these issues.

Case 2: Blunt force injury is one of the major categories of mechanical injury. Blunt force injuries are among the most common injuries sustained by persons. These injuries include abrasions (scrapes), contusions (bruises), and lacerations (tears). Blunt force is also a substantial component of chop wounds, injuries caused by relatively heavy-edged objects such as a machete or axe. Multiple factors and mechanisms are involved in injuries and deaths involving blunt forces. Understanding and evaluating injuries and deaths in which blunt force injuries may have played a role requires basic knowledge of injuries caused by blunt forces and how to distinguish them from other types of trauma; recognition of patterned injuries; and, recognition of injury patterns (e.g., pattern of falling versus pattern of being struck by an object). This lecture will provide a comprehensive review of these issues.

Case 3: Following cessation of life, the human body undergoes a variety of progressive changes leading to its ultimate breakdown. Intrinsic (autolysis and putrefaction) and extrinsic (environmental conditions, animal/insect activity, and funeral/burial procedures) factors affect the time course of this process and the appearance of the body/tissue. Proper evaluation of postmortem changes may be helpful in estimating the time since death and evaluating accuracy and reliability of other investigative information. Postmortem changes must be distinguished from antemortem disease/injury, may mask or obliterate pre-existing disease/injury, and may affect the performance, reliability, and interpretation of laboratory analyses. This lecture will review the postmortem changes, their causes and appearances, their proper recognition, their significance, and the usefulness and limitations of utilizing this information in a medicolegal death investigation.

Case 4: The death of an apparently healthy infant is a devastating event for the infant's survivors and is accorded significant attention by society. Infant death may be the caused by a wide variety of diseases and injuries, involve a variety of mechanisms, and can be natural, accidental, or homicidal. External and/or internal evidence of disease or injury may be lacking. Accurate recognition of the cause, mechanism, and manner of death has important implications for the survivors, other interested investigative and health agencies, and society in general. Recognition of factors involved in sudden unexpected infant deaths can help in enhancing the safety of other family members and serve as a basis for formulating death prevention strategies. This lecture will discuss the investigation and interpretation of findings in sudden unexpected deaths involving infants.

Case 5: Head injury is a common cause of trauma-related morbidity and mortality among children. Childhood head injury is not simply a smaller version of adult head injury because of significant differences between adults and children. Findings in serious head injury depend on the mechanisms of injury, duration of survival, age of the child, and, in some cases, co-morbid conditions. Proper evaluation of pediatric head injury is important in recognizing how an injury was sustained, excluding various potential or alleged mechanisms, evaluating accuracy and reliability of witness accounts, and aid in identifying the perpetrator in those cases involving inflicted injury. This lecture reviews the features of the pediatric head, mechanisms of injury, manifestations of head injury, and the interpretation of anatomic and clinical findings in the context of a medicolegal death investigation and quality of evidence in the literature.

Forensic Pathology, Death Investigation, Forensic Examination

H71 Insights Into the Postmortem Redistribution (PMR) of Diazepam, Methadone, and Morphine: Sampling Site, Time, and Method Matter

Eric Lemaire, MD, Medico-legal Institute University of Liège, Rue Dos-Fanchon 37, Liège B-4020, BELGIUM; and Carl J. Schmidt, MD, Wayne County MEO, 1300 Warren, Detroit, MI 48207*

After attending this presentation, attendees will understand that the site of sampling, the technique used, and the postmortem interval influence measured postmortem drug concentrations.

This presentation will impact the forensic science community by illustrating that popliteal blood yields lower drug concentrations than femoral blood samples and may more closely approximate antemortem drug concentrations. Also, sampling techniques and postmortem interval are associated with blood concentration variations of importance.

Fifty-seven cases were sampled as follows: Subclavian Blood – Dissection/Clamp (SBDC) technique; Subclavian Blood – Blindstick (SBBS) technique; Femoral Blood – Dissection/Clamp (FBDC) technique; Femoral Blood – Blindstick (FBBS) technique; Intracardiac Blood (ICB); and Popliteal Blood (PB). Cardiac blood was sampled in the right auricle after chest dissection. Popliteal blood was sampled after dissection and clamping of the popliteal vein because of its small caliber and depth in the popliteal fossa.

In 30 cases, blindstick and dissection/clamping were used at subclavian and femoral sites at the same time. In 27 cases, two samples were performed on the same body after a known time interval, alternating the sampling method.

Cases were divided in three groups: Group 1 (both methods), Group 2 (dissection/clamping), and Group 3 (blindstick).

To assess Postmortem Redistribution (PMR) for each substance and for each group, mean concentrations ratios were calculated as follows: [cardiac]/[subclavian], [cardiac]/[femoral], [cardiac]/[popliteal], [subclavian]/[femoral], [subclavian]/[popliteal], and [femoral]/[popliteal]. Ratios were compared between groups 2 and 3 to assess the difference between a blindstick and vein dissection.

Time-dependent variation of blood concentration and ratios was assessed two ways. One was to look at correlation between concentrations ratios and estimated postmortem interval (all cases). The second was to look at differences in concentrations in two samples taken after a time interval in the same case (27 cases).

Results indicate that the popliteal site appears to be less subject to PMR as seen in the mean concentrations ratios [femoral]/[popliteal] in Group 1: diazepam (N=24, mean ratio=1.45, $p < 0.001$); methadone (N=60, mean ratio=1.36, $p < 0.001$); morphine (N=49, mean ratio=1.49, $p < 0.001$).

Mean [femoral]/[popliteal] ratios tend to differ depending on sampling technique, except for morphine: Group 2: diazepam (N=20, mean ratio=1.13, p=0.097); methadone (N=38, mean ratio=1.25, $p < 0.001$); morphine (N=33, mean ratio=1.44, $p < 0.001$; Group 3: diazepam (N=18, mean ratio=1.95, p=0.0004); methadone (N=46, mean ratio=1.41, $p < 0.001$); morphine (N=33, mean ratio=1.43, $p < 0.001$).

Estimated postmortem interval appeared to increase mean [femoral]/[popliteal] ratios except for morphine (diazepam: r=0.61, p=0.0017; methadone: r=0.56, $p < 0.0001$; morphine: r=-0.029, p=0.85). Sampling interval appeared to affect methadone and morphine subclavian concentrations (methadone: difference=+124.06, p=0.009; morphine: difference=+31.00, p=0.042) whereas mean concentrations were not significantly different at the popliteal site for all substances.

This study is the first to evaluate concurrently three aspects of PMR in three selected drugs. Popliteal blood mean concentrations are significantly lower than femoral blood, a commonly used site for peripheral sampling, for all substances. Sampling method appears to have an effect, since [femoral]/[popliteal] mean ratios were lower when using the dissection/clamp technique. Postmortem interval also showed significant influence on mean subclavian blood concentrations and on [femoral]/[popliteal] ratios, suggesting that PMR is a progressive phenomenon in central and peripheral compartments, but popliteal blood seems more resistant to it.

Postmortem Redistribution, Sampling Techniques, Postmortem Interval

H72 Postmortem Distribution and Detection of Butyryl Fentanyl

Meghan S. Kessler, DO, 900 W Baltimore Street, Baltimore, MD 21223; Rebecca Jufer Phipps, PhD, State of MD, OCME, 900 W Baltimore Street, Baltimore, MD 21223; Meghan A. Mulligan, MS, 438 Madison Drive, Shrewsbury, PA 17361; Barry S. Levine, PhD, OCME, 900 W Baltimore Street, Baltimore, MD 21223; Russell T. Alexander, MD, OCME, 900 W Baltimore Street, Baltimore, MD 21201; Mary G. Ripple, MD, 900 W Baltimore Street, Baltimore, MD 21223; and David R. Fowler, MD, OCME, 900 W Baltimore Street, Baltimore, MD 21223*

After attending this presentation, attendees will have a better understanding of butyryl fentanyl analysis and postmortem concentrations.

This presentation will impact the forensic science community by providing medical examiners and toxicologists with information concerning an analytical method and postmortem concentrations for butyryl fentanyl.

Butyryl fentanyl (N-phenyl-N-[1-(2-phenylethyl)-4-piperidinyl]-butanamide, monohydrochloride) is a potent synthetic fentanyl analog with lower affinity for the μ-opioid receptor than fentanyl. While fentanyl is 54 times more potent than morphine, butyryl fentanyl remains at least seven times more potent than morphine. Butyryl fentanyl is not currently a scheduled drug under the Controlled Substances Act but may be considered a "controlled substance analog" due to its structural similarity to fentanyl. Neither fentanyl nor butyryl fentanyl is metabolized to the other and they are separate chemicals. Fentanyl analogs are increasingly more common, including acetyl fentanyl, which has been implicated in intoxication deaths nationwide. Butyryl fentanyl represents an emerging fentanyl analog relatively new to the United States and the Drug Enforcement Administration (DEA) reports that no cases have been identified in the Baltimore area prior to the one reported in this abstract and only four cases have been reported over the last several years in the United States. The State of Maryland Office of the Chief Medical Examiner (OCME) recently identified butyryl fentanyl in an inspection performed on June 25, 2015, of an overdose death where the cause of death was mixed drug (fentanyl, butyryl fentanyl, morphine, and alprazolam) intoxication and the manner of death was accident. The decedent was a 47-year-old White female found unresponsive by her son at home on June 22, 2015, at 3:15 a.m. She had a past medical history of lupus, high blood pressure, hepatitis C infection, hepatocellular carcinoma, gastrointestinal hemorrhage, and chronic back pain. Reportedly, she had not seen a physician in more than six months and would obtain drugs off the street for her pain control. An admission hospital urine drug screen on June 22, 2015, at 4:48 a.m. was positive for benzodiazepines and opiates and confirmation testing of the urine revealed an alpha-hydroxyalprazolam concentration of 870ng/mL and a morphine concentration of 2,400ng/mL. She spent three days in the intensive care unit at the hospital before being declared brain dead.

Butyryl fentanyl was identified at the OCME in an alkaline drug screen, which involved an alkaline liquid-liquid extraction of specimens followed by detection with Gas Chromatography — nitrogen-phosphorus detection and confirmation by gas chromatography — Mass Spectrometry (GC/MS). Butyryl fentanyl eluted after fentanyl on an HP-5 column and prominent GC/MS ions were 259, 189, and 146. Quantitation of butyryl fentanyl was performed using solid phase extraction followed by GC/MS. Briefly, internal standard (d5-fentanyl) was added to 1.0mL case specimen which was mixed with 2mL deionized water and 2mL of phosphate buffer (100mM, pH 6.0), centrifuged, and applied to conditioned CEREX® Trace-B columns. The columns were then washed with deionized water, 100mM acetic acid, and methanol and dried for five minutes at 50psi. Analytes were eluted with 3mL of 78:20:2 methylene chloride:isopropanol:ammonium hydroxide. Eluents were evaporated to dryness, reconstituted in 50μL ethyl acetate, and injected into the GC/MS. The GC/MS was operated in the selected ion monitoring mode, monitoring 259 (quantitation ion), 189, and 146 for butyryl fentanyl and 250 (quantitation ion) and 194 for d5-fentanyl. The limit of quantitation was administratively set at 5.0ng/mL and the method was linear to 400ng/mL. Butyryl fentanyl concentrations (ng/mL) in the case specimens are summarized below.

Postmortem Heart Blood	34
Postmortem Subclavian Blood	26
Postmortem Vitreous Humor	13
Antemortem Hospital Blood	40 (6/22/15 @ 04:45) & 32 (6/22/15 @ 20:20)
Additional toxicology findings in this case	Postmortem Heart blood: Diphenhydramine: 700ng/mL Promethazine: 70ng/mL Fentanyl < 5ng/mL Alprazolam: < 25ng/mL Antemortem Hospital Blood: Free Morphine: <10ng/mL (6/22/15 @ 04:45)

Butyryl fentanyl is less potent than fentanyl and its concentrations were noted to be higher than typical fentanyl intoxications, but lower than the acetyl fentanyl intoxications investigated by the Maryland OCME. In addition, the postmortem blood concentrations are likely affected by the three-day interval between drug use and death in this case. This is the first reported

case of butyryl fentanyl intoxication in the State of Maryland. The rarity of this new analog emphasizes the importance of recognizing emerging new drugs in postmortem toxicologic analysis.

Butyryl Fentanyl, Fentanyl, Toxicology

H73 Buprenorphine Prevalence in the Office of the Chief Medical Examiner (OCME) Cases Positive for Drugs of Abuse: To Screen or Not to Screen?

Diana Geli, 900 West Baltimore Street, Baltimore, MD 21223; Rebecca Jufer Phipps, PhD, State of MD, OCME, 900 W Baltimore Street, Baltimore, MD 21223; Meghan A. Mulligan, MS, 438 Madison Drive, Shrewsbury, PA 17361; Mary G. Ripple, MD, 900 W Baltimore Street, Baltimore, MD 21223; and David R. Fowler, MD, OCME, 900 W Baltimore Street, Baltimore, MD 21223*

After attending this presentation, attendees will be aware of the debate on the safety of buprenorphine as it relates to the actual prevalence of use and abuse in a drug-positive population.

This presentation will impact the forensic science community by increasing the awareness of the prevalence of buprenorphine in autopsy cases positive for drugs of abuse in Maryland and provoking thought on the necessity and effect of its inclusion into the initial postmortem toxicology drug screen and its impact on public health.

Buprenorphine is a partial opioid agonist approved by the U.S. Food and Drug Administration in 2002 for use in opioid addiction therapy. Buprenorphine is generally considered to have a more favorable safety profile relative to other opioids due to its ceiling effect and partial agonist effect. In higher doses, buprenorphine may actually block the effects of full opioid agonists and can potentially induce withdrawal in opioid-addicted individuals. Buprenorphine is available as a sole-active ingredient preparation as well as in combination formulations with naloxone to discourage misuse and diversion. Despite this, the drug continues to be misused as a self-medication for opioid withdrawal. Though relatively safe when used appropriately, multiple deaths have been reported due to buprenorphine use and misuse alone or in combination with other drugs. In several of the reported cases, the levels of buprenorphine were not considered toxic; however, since no other identifiable causes of death could be found, buprenorphine toxicity was still listed as contributory to the cause of death. In the majority of these cases, buprenorphine was taken with other drugs. Due to the rising number of individuals in Maryland receiving buprenorphine as part of their substance abuse treatment, the issue of its diversion and misuse in the drug abusing population has been a growing public health concern. There are currently 561 physicians in Maryland that have received waivers to prescribe buprenorphine for opioid addiction therapy. In 2014, there were 252,664 prescriptions written for buprenorphine in Maryland. Therefore, it is important to try to elucidate the drug's possible role in the death of individuals who use and/or misuse it, with or without the concomitant use of abused drugs. The recent increased availability of buprenorphine has fueled concern from certain groups of the possible role of buprenorphine in drug intoxication deaths involving other drugs of abuse. Therefore, the OCME performed buprenorphine screening of blood for cases testing positive for drugs of abuse from December 2013 to July 2014 to determine its prevalence in these cases.

Buprenorphine screening was performed by Enzyme-Linked Immunosorbent Assay (ELISA) with the Immunalysis™ Buprenorphine Direct ELISA kit. Screening was performed according to manufacturer's instructions with a blood cutoff concentration of 0.5ng/mL. From December 2013 to July 2014, a total of 2,744 autopsies were performed and 614 cases positive for drugs of abuse were selected for buprenorphine screening. Of the 614, only 30 cases (4.88%) screened positive for buprenorphine in blood. Of the 30 cases, confirmation was completed for five cases, resulting in four positive cases and one negative case. Confirmation in the remaining 25 cases is ongoing. Of the four confirmed positive cases, buprenorphine was a contributing factor in the cause of death in only three. In all three, additional drugs were present in blood, including ethanol, oxycodone, benzodiazepines, quetiapine, zolpidem, and amphetamine.

Given the increasing role of buprenorphine in opioid dependence therapy and the rising increase in the numbers of diverted buprenorphine being seized by law enforcement, concern for its role in the deaths of cases positive for drugs of abuse is expected; however, considering its pharmacological attributes, it is actually much safer in overdose than opioid full agonists. When used in combination with naloxone, it may discourage misuse and diversion. While the use, misuse, and diversion of buprenorphine is on the rise, its low prevalence in autopsy cases positive for drugs of abuse suggests that its actual role in contributing to death in this population may be very small. Its inclusion into initial postmortem toxicology screens may be unwarranted. Medical examiners should use caution in implicating buprenorphine as a cause of death in cases where non-toxic levels are detected in the absence of additional significant toxicological findings and no other cause of death is identified.

Buprenorphine, Screen, Public Health

H74 Chi-Squared Automatic Interaction Detection (CHAID) Analysis as a Technique for Discerning Patterns of Drug Use in Postmortem Toxicology

Candace Savonen, BS, Michigan State University, 108 Giltner Hall, 293 Farm Lane, East Lansing, MI 48825; Carl J. Schmidt, MD, Wayne County MEO, 1300 Warren, Detroit, MI 48207; and Michael Bannon, PhD, Wayne State University School of Medicine, 540 E Canfield, Detroit, MI 48201*

After attending this presentation, attendees will understand how a statistical technique, CHAID, can help discern drug use patterns and their effect on drug-related mortality in a death investigation setting. CHAID analysis is one of several decision-tree methods used to explore data and identify retrospective as well as predictive patterns. It has been used for data analysis in other medical fields.

This presentation will impact the forensic science community by helping find patterns of drug use in postmortem toxicology that are not evident in any other way. Recurrent analysis of toxicology data with CHAID may also assist in identifying changing patterns of drug use in a community.

Another aspect of these deaths is that there are no well-defined criteria for certification. Essentially, death certification in a drug-related case is a process of elimination in which, if no pathologic changes are found, what remains is a drug concentration which may or may not have diagnostic certainty but is known to be associated with an increased risk of death. Procedures like CHAID can be used to understand patterns of death certification and discerning implicit criteria that may not be obvious from the decision-making process when looking at these deaths.

All of the deaths in the Wayne County Medical Examiner's Office from December 2011 to December 2014 with any positive drug result were reviewed (4,297 cases). There were 284 compounds in the extended drug screen used. Test results were analyzed as binomial variables (i.e., a positive or negative result). Positive results for metabolites were combined with positive results for the parent compound. All cases with a positive toxicology result were included, including homicides, accidents due to trauma, suicides, and natural deaths and were useful for comparison. Cases with an unknown cause of death were excluded (six cases).

Data analysis was done using Microsoft® Excel® and IBM® Statistical Package for the Social Sciences (SPSS); the latter was used for generating decision trees. All compounds present in ten or more cases were included as independent variables in the model input. The CHAID algorithm uses non-parametric testing of combinations of these categorical variables to create a model that best predicts the response variable. In this case, the response variable is the drug of abuse that resulted in accidental death, as determined by a forensic pathologist. To generate the tree, parent nodes must have at least 40 cases and child nodes at least 20 cases, and each must have a significant chi-squared statistic ($p < 0.5$). The algorithm also generates gain and index charts that help evaluate the nodes with the most statistically significant results for the response variable (the drug of abuse).

Results showed that a positive morphine result was the best predictor of drug abuse as a cause of death. In cases positive for morphine, the next best predictor of drug abuse was a positive result for codeine. Drug abuse cases that were positive for morphine were also more likely to be positive for either citalopram, an antidepressant, or alprazolam, a benzodiazepine. Drug abuse cases positive for codeine and morphine were also likely to be positive for citalopram. Cases negative for codeine and positive for morphine were instead associated with alprazolam. In cases negative for morphine, acetaminophen was the next best predictor for drug abuse. Acetaminophen-positive results were co-occurent with hydrocodone-positive results and negatively associated with methadone. Hence, cases of drug abuse in which morphine was negative were more likely positive for hydrocodone and acetaminophen or methadone use.

Until now, the pattern between morphine, citalopram, and alprazolam was unknown, as well as that of acetaminophen/hydrocodone and methadone. Methadone has an increased risk of drug-related death independent of other drugs based on this data set. The co-occurrence of acetaminophen and hydrocodone was not unexpected; they are often combined as a prescription, but their statistical co-occurrence is validation of the power of this analytical approach.

CHAID analysis can help discern patterns of drug use that were not previously apparent. Although currently in evaluation, it is likely that recurrent CHAID analysis of drugs in these deaths can help assess changing patterns of mortality associated with drug use.

Postmortem Toxicology, CHAID, Drug Abuse

H75 Deaths Associated With Synthetic Cannabinoids in Mississippi

Mark M. LeVaughn, MD, Office of the Chief Medical Examiner, 215 Allen Stewart Road, Pearl, MS 39208; Brent Davis, MD*, 1700 E Woodrow Wilson, Jackson, MS 39216; Lisa Funte, MD, Mississippi State Medical Examiner, 1700 Woodrow Wilson, Jackson, MS 39216; and Thomas Dobbs, MD, Mississippi Department of Health, 1700 Woodrow Wilson, Jackson, MS 39216*

After attending this presentation, attendees will understand the clinical presentation and autopsy findings of patients with synthetic cannabinoid toxicity.

This presentation will impact the forensic science community by reviewing the results of numerous cases of synthetic cannabinoid toxicity that occurred over a short period of time in Mississippi.

Mississippi has recently experienced the largest recorded outbreak of toxic events related to synthetic cannabinoids in the United States. Synthetic cannabinoids are commonly referred to as "Spice." These unregulated drugs are chemically unrelated to the psychoactive compounds of marijuana and have highly variable and unpredictable physical effects. Synthetic cannabinoids are usually sprayed onto plant material and smoked in a similar fashion as marijuana. There are multiple street names such as "K2," "Spice," "Scooby Snacks," "Mojo," and "Anthrax." There are numerous forms of synthetic cannabinoids and many are chemically unrelated.

According to state statutes, any compound that contains any quantity of any form of synthetic cannabinoid is illegal in the state of Mississippi. In April of 2015, the University of Mississippi Medical Center Emergency Department reported to the Mississippi State Department of Health an unusual increase in the number of emergency room admissions related to the ingestion of synthetic cannabinoids or "Spice." The Mississippi Poison Control Center in collaboration with other hospital emergency rooms across the state identified 1,243 patients that presented to and were admitted to hospital emergency rooms during the months of April and May of 2015. During the same time period, numerous Mississippi county coroners from all regions of the state were referring deaths that were suspected to be due to the ingestion of "Spice" to the Mississippi State Medical Examiner's Office. There was a wide age distribution of these patients ranging from 12 years to 72 years of age with the majority between 18 years and 39 years of age. The majority of the patients were male (83%). Common symptoms included agitation, violent behavior, somnolence, confusion, tachycardia, and hypertension. Numerous patients were unresponsive and required admission to the intensive care unit. Blood and urine specimens were collected from hospital patients. Blood from multiple sites, urine, bile, and vitreous were collected from medical examiner cases. The body fluid samples, in addition to plant material when available, were sent to several reference toxicology labs. The laboratories were able to test for approximately 40 different synthetic cannabinoid compounds. MAB-CHMINACA, a recently recognized synthetic cannabinoid, was identified in the blood of multiple patients. A five-member team from the Centers for Disease Control and Prevention spent two weeks in Jackson, MS, with the objective of characterizing the outbreak, identifying associated deaths, determining the risk factors for adverse effects, and identifying the drug source to stop further spread of the outbreak.

In conclusion, the clinical and investigative information in addition to the autopsy and toxicology findings of 11 of these patients will be presented. The goal is to provide medical examiners with a better understanding of the autopsy and toxicology findings when dealing with patients who are suspected of dying as a result of ingesting synthetic cannabinoids.

Synthetic Cannabinoid, MAB-CHMINACA, Spice

H76 Using Enzyme-Multiplied Immunoassay Technique (EMIT) Analysis of Vitreous Humor to Identify Heroin Use at Autopsy

Brandi C. McCleskey, University of Alabama at Birmingham, 619 19th Street, S, Birmingham, AL 35249; C. Andrew Robinson, Jr., PhD, University of Alabama, Laboratory Medicine Division, Dept of Pathology, Birmingham, AL 35233-7331; and Daniel W. Dye, MD, Jefferson County Coroner/Medical Examiner Office, 1515 6th Avenue, S, Rm 220, Birmingham, AL 35233*

After attending this presentation, attendees will be aware of the potential utility of vitreous humor screening for 6-Acetylmorphine (6-AM) in cases of suspected heroin use with negative urine and/or blood screens for 6-AM and opiates.

This presentation will impact the forensic science community by stressing the importance of scene investigation and knowledge of circumstances surrounding death for proper analysis of specimens collected during postmortem examination.

Deaths due to the toxic effects of heroin have risen more than 286% in the past decade. The rise in use of heroin among most demographic groups is likely contributed to by the insurgence of opioid painkiller misuse and addiction.[1] Medical examiner offices are faced with cases in which heroin use is suspected given scene investigation findings and eyewitness accounts of activities prior to death. Toxicological evidence is often utilized to support the physical and postmortem findings; however, given the many factors that affect the breakdown of heroin *in vivo*, screening tests for heroin's unique metabolite 6-AM or opiates in blood or urine may be negative, leading to a possible misdiagnosis.

Utilizing detection methods to identify 6-AM is often useful for the forensic pathologist in determining cause of death and, in some cases, is supportive of time of survival after lethal injection of heroin.[2] Vitreous humor is a readily available biologic specimen in most forensic postmortem examinations. Heroin is metabolized via deacetylation to 6-AM, which undergoes further esterase conversion to morphine. This metabolism occurs quickly in the blood where esterase activity is high. The lipophilicity of heroin makes its transfer across the vitreous-blood barrier favorable where it is rapidly metabolized to 6-AM in the vitreous humor. Furthermore, the window of detection for 6-AM is extended due to the minimal esterase activity present in the vitreous. Vitreous levels of 6-AM likely indicate recent exposure to heroin prior to death even in the absence of blood and urine 6-AM detection.

Five cases from the Jefferson County Coroner/Medical Examiner Office in 2014 were identified in which the decedent was suspected of using heroin, given the history and scene investigation findings prior to death, but postmortem urine drug screens were negative for opiates and 6-AM. The vitreous humor was then screened for 6-AM using EMIT and 6-AM was subsequently detected in four to five cases (80%). In all five cases, morphine was detected and quantified in the blood. These combined results supported the scene investigation and physical findings of the postmortem examination, including needle tracks and puncture sites, consistent with heroin toxicity.

Negative toxicology is often a finding in delayed death cases and complicates the cause-of-death designation. The prolonged window of detection of 6-AM in the vitreous humor may allow further analytical evidence of heroin use and toxicity. Although advanced methods have been evaluated in the literature for vitreous humor opiate testing, the common and relatively simple method of EMIT typically utilized for urine drug screening can also provide an additional screening method using vitreous humor in selected cases.[3]

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Heroin, Vitreous, Autopsy

H77 Post-Traumatic Meningitis in the Setting of an Accidental Fall of a Two-Year-Old Child

Krishna D. Shah, MD, UK Department of Pathology, 800 Rose Street, MS-117, Lexington, KY 40536; Sarah A. Higdon, MD, University of Kentucky, 3344 Orchard Grass Road, Lexington, KY 40509; Meredith H. Frame, MD, Office of the Associate Chief Medical Examiner, 100 Sower Boulevard, Ste 202, Frankfort, KY 40601; and Gregory J. Davis, MD, UK Medical Center, MS 117, 800 Rose Street, Lexington, KY 40536-0298*

This goal of this presentation is to describe a case of bacterial meningitis that demonstrates an unusual complication of blunt force injuries that should be considered in the differential diagnosis.

This presentation will impact the forensic science community by discussing a rare complication of head trauma and the importance of correlating the clinical and radiological findings (when available) with the anatomic findings at autopsy.

This case example highlights a rare but serious and potentially fatal complication of head injury-bacterial meningitis. Medical examiners should consider this possibility, as the disease may not be recognized clinically or the decedent may not seek medical evaluation prior to death.

A previously heathy two-year-old male fell approximately ten feet from a set of bleachers, landing prone on the ground. He did not lose consciousness and, aside from facial bruising and edema, had no changes in behavior or mentation. He was examined the following morning at a local hospital, where his neurologic exam was normal, and was discharged home. Later that evening, he became irritable and lethargic with nausea, vomiting, and decreased urine output. The following morning (two days post-fall), he was taken to another hospital and found to have a temperature of 102°F. A Computed Tomography (CT) scan of the head and face/sinus without contrast showed a questionable frontal skull fracture.

Neurosurgery was consulted and initially suspected a post-concussive syndrome. The boy was admitted to the pediatric care unit. On hospital day two, he became somnolent, unresponsive to verbal or physical stimuli, and was found to have asymmetric pupils. He was intubated, and a stat Magnetic Resonance Imaging (MRI) showed diffuse leptomeningeal enhancement suggesting meningitis, possibly secondary to a basilar skull fracture. He was started on broad-spectrum antibiotics. A lumbar puncture was not performed due to concern for herniation. His condition continued to worsen over the next several days and brain death was confirmed on hospital day five. The decision was made to withdraw care and the coroner was notified of his death. He was transported to the medical examiner's office for autopsy.

The external examination was significant for scattered abrasions of the face, as well as periorbital hematoma. Upon internal examination, there was marked cerebral edema and uncal herniation. The leptomeninges were cloudy with yellow/green exudate. The frontal and parietal-occipital lobes of the left cerebrum showed focal contusion. Fractures were identified in the left anterior basilar skull with associated focal hemorrhage.

Cerebrospinal Fluid (CSF) collected postmortem showed 85% neutrophils, glucose=2mg/dL, and tested positive for the *Streptococcus pneumoniae* antigen (*S. pneumoniae* was also identified in antemortem blood cultures drawn at the hospital on admission). Microscopic examination showed a dense acute inflammatory infiltrate of the leptomeninges, vascular congestion, and neuronal cell death. Gram positive cocci were identified on the Gram stain. The cause of death was complications of pneumococcal meningitis due to basilar skull fractures due to a fall from approximately ten feet. The manner of death was determined as accident.

The incidence of bacterial meningitis due to basilar skull fracture is 3%. Mechanism of infection includes direct invasion of CSF by bacteria that may normally colonize the nasopharyngeal tissue (i.e., CSF leaking from nose or ear that could lead to sepsis and cardiovascular compromise). Therefore, recognition of the signs of a basilar skull fracture is important. Most cases will have positive CSF cultures and blood cultures. The most common organisms are *S. pneumoniae* (as in this case), Group A *Streptococcus*, and *Haemophilus influenzae*. Despite the increased risk of bacterial meningitis, it is still considered controversial to administer prophylactic antibiotics to this population; no major study has definitively proved benefit. The mortality and morbidity rate in children with pneumococcal meningitis are approximately 10% and 30%, respectively.

It is important for medical examiners to consider this rare complication in decedents with a history of head trauma as the diagnosis may be missed or be uncertain clinically, or the decedent may not seek medical evaluation.

Meningitis, Accident, Skull

H78 Defining "Mass Fatality Incident" for Medicolegal Jurisdictions in the United States: A Planning Tool

Allison Woody, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; Jason M. Wiersema, PhD, Harris County Institute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054; Frank DePaolo, BS, NYC Office of Chief Medical Examiner, 520 First Avenue, Rm 123, New York, NY 10016; Emily Carroll, 421 E 26th Street, New York, NY 10016; Adriana M. Fernandez, BS, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; and Rachel Canfield, BA, 103 Fenway Loop, #104, San Marcos, TX 78666*

The goal of this presentation is to provide a summary of the survey data collected and analyzed as part of a joint project between the Harris County Institute of Forensic Sciences and the Office of the Chief Medical Examiner of the City of New York. Preparedness is imperative for medicolegal jurisdictions of all sizes to ensure continuity of operations following a mass fatality incident that exceeds local capacity. The Disaster Victim Identification (DVI) Subcommittee of the Organization of Scientific Area Committees (OSAC) and other entities are focused on the development of best-practice guidance for the management of mass fatality incidents, but there is little empirical data that can be used to inform local application of best practices or planning initiatives.

This presentation will impact the forensic science community by providing a tool for medicolegal jurisdictions to evaluate local risk for mass fatality incidents.

The project was intended to: (1) generate a realistic definition of the term "mass fatality" for local jurisdictions that are in the process of developing mass fatality incident response plans; and, (2) provide a risk-assessment tool for mass fatality incident susceptibility research. Local jurisdictions often seek guidance for mass fatality planning from federal initiatives that are often associated with emergency management grant programs (such as Urban Area Security Initiative (UASI), State Homeland Security Program (SHSP), and Regional Catastrophic Preparedness Grant Program (RCPGP)) that are intended to provide preparedness benchmarks for large emergency management jurisdictions located in large urban areas. These programs are typically focused on preparations for catastrophic man-made or natural incidents that involve large numbers of decedents in complicated environments. This guidance is often of minimal utility for medium or small jurisdictions with limited or no mass fatality response capabilities. The need remains for a tool that can be utilized by jurisdictions of variable size and capability to determine their risk and thus develop appropriate preparations to respond to mass fatality incidents.

To develop this tool, data were gathered for all multiple fatality incidents that occurred in the United States between January 1, 2000 and December 31, 2014 that resulted in four or more fatalities. For each incident, the date, city, county, state, number of fatalities, incident type, incident subtype, and incident category were recorded. Incident type is a record of whether an incident was man-made or natural, and incident subtype is a record of whether the incident type is accidental, weather related, criminal, etc. The database also includes a brief description of the incident itself (bridge collapse, mass shooting, etc.), the population of the jurisdiction in which the incident occurred, whether a manifest was available, and whether the incident response included a federal disaster declaration. These data were extracted from federal databases including the National Transportation Safety Board (NTSB), National Oceanographic and Atmospheric Administration (NOAA), the Mine Safety and Health Administration (MHSA), the Bureau of Safety and Environmental Enforcement (BSEE), the National Incident-Based Reporting System, and the Fatality Analysis Reporting System (FARS). Single incidents affecting multiple jurisdictions that resulted in fatalities were split by county/city to avoid over-representing the burden of an incident on a single medicolegal jurisdiction.

A total of 2,934 incidents met the criteria for inclusion in the database, accounting for more than 19,000 fatalities. The average number of fatalities per incident during the 15-year period included in the sample is seven. There is significant variability in the frequency of incidents by location and incident type, but the average number of fatalities is less variable by location (with the exception of the few locations within which very large incidents occurred (New York City and New Orleans)). The median number of fatalities in these areas resembles the median number in other areas. The presentation will include Geographic Information Systems (GIS) mapping of the results to provide a more detailed illustration of the incident distribution. The end result is a valuable interactive planning tool that provides preparedness benchmarks and a scalable strategy for local medicolegal jurisdictions.

Mass Fatality, Disaster Victim Identification, Preparedness

H79 Unexpected Pediatric Death Due to Congenital Mesenteric Defect

Hannah Claire Jarvis, MRCS, Montefiore Medical Center, 111 E 210th Street, Bronx, NY 10467; and Carolyn A. Kappen, MD, Office of Chief Medical Examiner, City of New York, 520 First Avenue, New York, NY 10016*

After attending this presentation, attendees will understand that a congenital transmesenteric hernia is an uncommon cause of intestinal obstruction that can be rapidly fatal. With careful dissection of the gastrointestinal tract at autopsy, the underlying etiological diagnosis and cause of death can be identified and documented.

This presentation will impact the forensic science community by highlighting an unexpected pediatric death due to a congenital transmesenteric hernia. Ascertaining the etiology of the necrotic bowel at autopsy is often difficult but pertinent in determining the cause and manner of death.

Transmesenteric hernia is a rare form of internal hernia through an acquired or congenital defect in the mesentery. It most commonly affects the small bowel and can result in intestinal obstruction with subsequent incarceration and strangulation that can be rapidly fatal. Although an uncommon entity, the mortality rate in untreated cases with gangrenous bowel can reach 80% and potentially even higher.[1,2] This report is of an unexpected pediatric death due to a congenital mesenteric defect.

A six-year-old Hispanic boy with no past medical history presented to the emergency department from home via ambulance in cardiac arrest. The previous day, the child's mother had taken him to the pediatrician with complaints of rhinorrhea and multiple episodes of vomiting. He was diagnosed with a viral syndrome and sent home with an oral electrolyte solution. The next day, the child continued to vomit and became unresponsive. Upon arrival in the emergency department, the child was noted to have dry mucosa and a mildly distended abdomen. The Pediatric Advanced Life Support (PALS) protocol was followed without success. The death was reported to the medical examiner's office, as the cause of death was unclear.

At autopsy, the child was well developed and external examination was unremarkable. The esophagus and stomach were unremarkable except for focal gastritis. At 70 inches distal to the ligament of Treitz, there was a sharp demarcation in the small bowel, from a normal tan-pink color to dark and necrotic. This necrotic segment of bowel was 76 inches long. Distal to this was viable small and large bowel extending to the distal rectum. Near the ligament of Treitz, there was a one-half inch oval-shaped defect in the mesentery through which three loops of necrotic small bowel protruded. Histological examination demonstrated transmural necrosis without inflammation. Postmortem cultures were non-contributory. No drugs were detected in a routine toxicological panel. The cause of death was incarcerated internal hernia with entrapped small intestine due to congenital mesenteric defect. The manner of death was natural.

A transmesenteric hernia is a rare cause of intestinal obstruction and death. First documented by Rokitanksky in 1836, the incidence of internal hernias is estimated to be 0.2%-0.9%.[3,4] Congenital transmesenteric hernias comprise 35% of congenital internal hernias.[5] Patients may be asymptomatic or present with non-specific misleading symptoms, particularly in elderly and pediatric patients, ultimately leading to an unexpected fatality. Radiological and laboratory studies are minimally helpful as there are no specific findings.[6] Transmesenteric hernias are difficult to diagnose because their appearance and location can be variable. These internal hernias lack a hernia sac and, therefore, can present anywhere in the peritoneal cavity.[7] Acquired transmesenteric defects usually present in adults as a result of surgical manipulation of the bowel and mesentery, such as in a Roux-en-Y gastric bypass or from blunt abdominal trauma. Ascertaining the etiology of the necrotic bowel at autopsy is often difficult but pertinent in determining the cause and manner of death. On opening the abdomen, there is usually free fluid, which may be blood-stained or dark in the presence of a strangulation. The bowel can be gangrenous or perforated, resulting in fecal contents or exudate in the abdominal cavity. The mesenteric defect is usually located close to the ligament of Treiz or the ileocaecal valve, and is typically ~one inch in diameter.[8] Careful, meticulous dissection, along with photographs, will help identify and document the underlying etiology and cause of death.

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Transmesenteric Hernia, Pediatric, Bowel Obstruction

H80 Entrance or Exit? A Multidisciplinary Approach to Gunshot Wound Interpretation on Fresh Remains

MariaTeresa A. Tersigni-Tarrant, PhD, Saint Louis University School of Medicine, Center for Anatomical Science, 1402 S Grand Boulevard, M306, St. Louis, MO 63104; Deiter J. Duff, MD*, University of Missouri, One Hospital Drive, M263 Med Sci Building, Columbia, MO 65212; and Jane W. Turner, PhD, MD, St Louis City MEO, 1300 Clark Street, St. Louis, MO 63103*

The goal of this presentation is to provide attendees with information on the advantages of using a multidisciplinary approach to the assessment of gunshot wound trauma on fresh remains at autopsy.

This presentation will impact the forensic science community by providing an example of how advantageous it is for forensic pathologists and anthropologists to work together when assessing gunshot wound trauma on fresh remains at autopsy. The use of both well-trained practitioners can indeed enhance the assessment of gunshot wound trauma and help to clearly identify entrance and exit wounds even when these wounds appear atypical in nature. This can be of particular importance when there are possible multiple shooters and also in corroborating witness accounts.

This case involves a child who was shot while inside a vehicle with a single perforating gunshot wound of the chest. Scene investigation revealed that gunshots were coming from nearly opposite directions and the child was caught in the crossfire, making the determination of direction of utmost importance. The bullet had passed through intermediary targets, causing atypical wound features. Evaluation of the direction of the shot was difficult based on evaluation of the skin because neither wound had clear characteristics of an entrance.

Given the unusual appearance of the entrance and exit wounds, an anthropological assessment of thorax was requested after autopsy. The anthropologist was blind to the circumstances of the case and was simply asked to assess entrance/exit trauma to help determine the direction of the shot. Trauma was identified on the right ribs seven trough ten during the *in situ* analysis. The surrounding soft tissue was removed using a scalpel. Bony trauma was noted on right ribs nine and ten, with possible bony damage noted on right ribs seven and eight. Photographs were taken of the bony damage *in situ*. Right ribs seven through ten were then removed using a scalpel and retained for further analysis. The vertebral bodies of thoracic vertebrae seven through ten were removed during autopsy due to the presence of bony damage on the right side of the 9th and 10th thoracic vertebral bodies; therefore, these elements were also analyzed and retained for further analysis.

The retained ribs and vertebral bodies were macerated using warm water to assist in the removal of adherent soft tissue. After warm water maceration, the remaining soft tissue was removed from the ribs using a soft-bristled brush and forceps. The ribs were then allowed to air dry. In an effort to preserve the anatomical relationship of the vertebral bodies, the remaining adherent soft tissue on the vertebral bodies was not removed. Instead, the vertebral bodies were analyzed as a unit and placed into formalin after analysis.

The retained skeletal remains of the decedent exhibit peri-mortem trauma to right ribs 9 and 10, as well as thoracic vertebral bodies 9 and 10 that is consistent with ballistic trauma. The characteristics of the ballistic trauma to the ribs and vertebral bodies suggest that the force occurred in a posterior-to-anterior direction.

Simultaneously, further autopsy examination revealed other evidence consistent with a posterior-to-anterior direction. A small amount of dark textile material was identified in the soft tissue of the entrance wound and in the perforated right lung nearest the entrance wound.

Two disciplines, forensic pathology and forensic anthropology, working together with scene investigators came together as a team to form a reliable interpretation. The forensic pathologists, if working alone, would have otherwise had difficulty determining the direction of the shot and may have ultimately listed the direction as indeterminate. This case illustrates the importance of a multidisciplinary approach in some cases of gunshot wounds even on fresh remains.

Pathology/Biology, Anthropology, Gunshot Wound Interpretation

H81 In-Custody Deaths in Sweden: 1992-2014

Susan Sprogoe-Jakobsen, Hoppets gränd 4, Umeå 903 34 Umeå, SWEDEN; Jonn Ekman, BM, Section of Forensic Medicine, PO Box 7616, Umeåå SE-907 12 Umeå, SWEDEN; and Anders Eriksson, MD, PhD, Umea University, Dept Forensic Medicine, PO Box 7616, Umea SE-907 12, SWEDEN*

After attending this presentation, attendees will have a better perception of how manner of death differs between short-time and long-time incarceration and of how deaths in custody may be prevented.

This presentation will impact the forensic science community by increasing knowledge regarding deaths of persons in custody in Sweden.

Jail and custody are controlled environments where the contents of the immediate surroundings as well as the supervision of the inmates are tightly regulated. Despite this, a number of deaths occur each year in these confined spaces. The purpose of this study was to investigate the characteristics of deaths in custody suites, jails, and prison in Sweden. Suicide methods were of specific interest, considering the obviously excellent possibilities of prevention.

In Sweden, there are three types of facilities used to incarcerate a person. The system for how these are used differs somewhat from that of other countries, and the definitions of "custody suite," "jail," and "prison" used in this presentation will be presented.

Using the database of the National Board of Forensic Medicine, all cases from 1992 through 2014 in which the words "police," "custody," "cell," "institution," "custody suite," and "prison" were used in the "site of death" box in the death certificate were scrutinized, generating a total number of 222 relevant cases. A complete review of the police reports, medical records, and the autopsy report was performed in cases from 2007 through 2014.

Among the 222 deaths in 1992-2014, there were 28 natural deaths, 77 accidental deaths, 116 suicides, and 1 homicide. Twenty-five out of the 28 accidental cases where data was available involved alcohol and/or licit or illicit drugs. Of the 27 accidental deaths where data was available, 22 occurred in a custody suite. In 11 out of 15 accidental deaths, the time of death was within four hours of incarceration.

In all but four suicides, hanging was the method used (n=50). Of the 54 suicides, 11 occurred in a custody suite and 43 in jail. In 27 out of 38 suicides, the deceased were found between 6:00 a.m. and 12:00 p.m. In all except four suicides from 2007 through 2014, hanging was the method used (n=50). In two cases, the method was suffocation, in one case the method was strangulation, and in one case the deceased had cut his throat. The annual suicide incidence increased from 4.13 (1992-2006) to 6.75 (2007-2014).

A clear distinction between custody suite and jail regarding the manner of death was observed, with accidental and natural deaths dominating the former and suicides most frequent in the latter.

In one-fourth of the accidental deaths (2007 through 2014), accidental intoxication *contributed* to death, with disease as the underlying cause of death. It is obviously important to distinguish between acute inebriation/intoxication and the deterioration of an already manifest disease.

The reason most suicides were discovered in the morning is probably because the inmates are alone and unsupervised during the night. The increased risk of suicide within the incarcerated population compared to the general population is consistent with studies from other countries. Although preventive measures were applied by the Swedish Prison and Probation Service in 2007, the annual incidence of suicide has not decreased, indicating that more effort regarding suicide prevention is needed. The reason for the increase cannot be accounted for in this study and demands further analyses.

Jail, In Custody, Deaths

H82 Case Report of Cerebral Tissue Pulmonary Embolism (CTPE) Following Blunt Force Head Injuries

Paul V. Benson, MD, UTHSC/Shelby County Medical Examiner, 637 Poplar Avenue, Memphis, TN 38105; and Cory Bosworth, BS, University of Tennessee Health Science Center, 90 Harbor Town Square, Apt 304, Memphis, TN 38103*

After attending this presentation, attendees will better understand CTPE, be able to identify the finding in similar cases, and understand its significance.

This presentation will impact the forensic science community by highlighting a rare complication of blunt force head injuries.

A case of an adult male who sustained blunt force head injuries and subsequent cerebral tissue pulmonary embolism is reported. The subject was found at the foot of his porch stairs. He was transported to the hospital, where he remained in critical condition for nearly 24 hours until his death. Toxicology indicated acute alcohol intoxication, and investigation of the circumstances leading up to the injury indicated a probable accidental fall from the top step to the ground. Examination showed blunt force injuries of the head including subdural hemorrhage and basilar skull fracture. At autopsy, multiple skull fractures and intracranial hemorrhages were observed, though the dura mater remained intact. Gross examination of the lungs showed no evidence of pulmonary embolism. Microscopic examination of the lung tissue showed multiple CTPE, confirmed by glial fibrillary acidic protein immunohistochemistry stain. In this case, the immunohistochemistry for glial fibrillary acidic protein showed variable positivity, likely due to necrosis of the embolized cerebral tissue.

Cerebral tissue pulmonary embolism is a rare complication of severe blunt force head injuries. It most often occurs in infants following injury associated with a difficult delivery. In adults, CTPE is always associated with severe blunt force injury of the head. CTPE is much less common in adults than in neonates. In one study, over a period of four years from 1989 to 1992, 102 head trauma fatalities were reported. Of those, only ten were discovered to have findings of brain tissue emboli, and only seven of those maintained an intact dura.[1] This indicates that the brain tissue does not need to travel through a large cerebral venous sinus, but can make its way to the lungs via the small meningeal or cerebral veins.

The clinical significance of cerebral tissue pulmonary emboli is variable. On one hand, some findings might be considered incidental, although even in these cases, the high concentration of thromboplastin found normally in neural tissue may contribute to the cause of death when it makes its way into the vasculature. When brain tissue comes in contact with the blood stream, it causes coagulation and shock. Therefore, even the so-called "incidental findings" may have been more contributory to the death of the patient than the term suggests. Conversely, in the cases of obvious macroscopic CTPE, death can be brought about much more directly by obstructing the pulmonary arteries.

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Head Trauma, Pulmonary Embolism, Cerebral Tissue

H83 A Retrospective Study of the Histologic Features and Scene Investigation in the Differential of Homicidal and Accidental Childhood Asphyxial Deaths

Theodore T. Brown, MD, 92 SW 3rd Street, #2606, Miami, FL 33130; Nicholas I. Batalis, MD, Medical Univ of South Carolina, 171 Ashley Avenue, Ste 309, MSC 908, Charleston, SC 29425; Joni L. McClain, MD, 15505 Daybright Drive, Edmond, OK 73013; Tracey S. Corey, MD, OCME, 810 Barret Avenue, 7th Fl, Louisville, KY 40204; Kim A. Collins, MD, LifePoint Organ and Tissue Donation Services, 3950 Faber Road, Charleston, SC 29405; Jeffrey M. Jentzen, MD, University of Michigan, 300 N Ingalls, NI2D19 - SPC 5452, Ann Arbor, MI 48109; and Joseph A. Prahlow, MD, Western Michigan University School of Medicine, 300 Portage Street, Kalamazoo, MI 49007*

After attending this presentation, attendees will understand that an autopsy and investigation of childhood asphyxial deaths can likely benefit from a better histologic examination.

This presentation will impact the forensic science community by narrowing the gap of uncertainty when certifying childhood asphyxial deaths.

Childhood asphyxial deaths, one of the leading causes of trauma deaths in this age group, present many challenges for forensic pathologists and investigators.[1]

Distinguishing between childhood homicidal and accidental asphyxial deaths requires an exhaustive investigation. While extensive external injuries may be present to raise the suspicion of homicidal asphyxia, often these injuries are minimal or absent. In addition, no set of histologic criteria is pathognomonic for asphyxial deaths, nor are there distinct findings to differentiate between homicidal and accidental deaths. Rather, the diagnosis is often based on scene investigation, including suspect and witness statements.

In an attempt to identify histologic features that can better aid the forensic pathologist in assigning the cause and manner of death, this pilot study looked at routine lung sections taken from childhood asphyxial deaths (ages three days to five years old). Hematoxylin and Eosin (H&E) and iron stains from 20 homicidal cases, 16 accidental cases, and 18 non-asphyxial cases (controls) were blindly assessed by three forensic pathologists. Eight specific lung histologic features were graded with a score from zero to four, based on published criteria.[2,3] The eight graded features include hemosiderin-laden macrophages, intraalveolar hemorrhage, intraalveolar hyperexpansion, pulmonary edema, septal/interstitial hemorrhage, interstitial edema, interstitial emphysema, and alveolar collapse.

Three histologic features (presence of hemosiderin-laden macrophages (average scores of 1.17, 0.42, and 0.74 for homicidal, accidental, and control cases, respectively; septal/interstitial edema (0.87, 0.44, and 0.26 for homicidal, accidental, and control cases, respectively); and interstitial emphysema (0.82, 0.42, and 0.57 for homicidal, accidental, and control cases, respectively)) were more prominent in homicidal asphyxia cases compared to accidental and control cases in the pilot study. While this data is not quite statistically significant between homicidal and accidental cases (p-values of 0.06, 0.06, and 0.08, respectively), the reviewers reliably demonstrated increased agreement on these three features. The five remaining histologic features did not demonstrate clear differences between homicidal, accidental, and control cases.

Due to the limited specific findings during an autopsy or investigation, childhood asphyxial cases continue to present many challenges to the forensic pathology community. Definitive evidence, scene investigation, and witness statements are needed to correlate with the autopsy findings. Currently, the only definitive evidence when assigning homicide as the manner of death is a confession by the suspect or credible witness statements, if a confession is not given. If neither a confession nor credible witness statements are given, pathologists and investigators must rely solely on their autopsy and scene findings to best determine the cause and manner of death. The same is true of accidental asphyxia.

The eight histologic features described here prompt further evaluation and comparison in a larger cohort to see if the presence of these specific features can help forensic pathologists when assigning the cause and manner of death. In addition, further studies are warranted to determine if particular autopsy techniques, such as inflating the lungs with formalin before cutting sections to be submitted for histologic evaluation, can help forensic pathologists better certify asphyxial deaths.

In summary, as one of the leading causes of traumatic deaths in children, evaluation of asphyxial deaths can likely benefit from a better histologic examination. This pilot study attempts to narrow the gap of uncertainty when certifying childhood asphyxial deaths.

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H84 Postmortem Iris Recognition and Its Application in Human Identification

Alora Sansola, Boston University School of Medicine, 72 E Concord Street, Boston, MA 02118; Dennis J. Chute, MD, Dutchess County MEO, 168 Washington Street, Poughkeepsie, NY 12601; Robert J. Bready, MS, Dutchess County MEO, 168 Washington Street, Poughkeepsie, NY 12601; and Amy N. Brodeur, MFS, Boston University School of Medicine, Biomedical Forensic Sciences, 72 E Concord Street, R806, Boston, MA 02118*

After attending this presentation, attendees will have a general understanding of iris recognition and how iris recognition technology can be used to locate and detect iris codes in postmortem globes (eyes).

This presentation will impact the forensic science community by demonstrating that iris scans collected from an individual at different postmortem time intervals can be identified as the same iris initially enrolled and by providing insight on how postmortem iris identification could be implemented in a forensic setting.

Iris recognition is a validated and non-invasive human identification method currently implemented for the purposes of surveillance and security (i.e., border control, schools, and the military).[1-6] Similar to Deoxyribonucleic Acid (DNA), irises are a highly individualizing component of the human body. Based on a lack of genetic penetrance, irises are unique between an individual's left and right iris and between identical twins, proving to be more individualizing than DNA.[4,7] At this time, little to no research has been conducted on the use of postmortem iris scanning as a biometric measurement of identification.

Research was conducted at the Dutchess County Medical Examiner's Office (DCMEO) in New York, NY. Only decedents with intact globes were analyzed and data collection was not limited based on a decedent's age, sex, race, or cause/manner of death. Initial iris scans were captured as soon as possible to minimize the Postmortem Interval (PMI) at the time of enrollment. Subsequent iris scans were collected periodically within 24 hours of the initial scan or until the decedent was no longer in the DCMEO custody.

Of the 43 cases involving 148 subsequent iris recognition scans, an 80% match rate was observed, demonstrating that iris recognition technology is capable of isolating and detecting an individual's iris code in a postmortem setting. A chi-square test of independence showed no significant difference between match outcomes and the globe scanned (left vs. right), and gender had no bearing on the match outcome. There was a significant relationship between an individual's iris color and match outcome, with blue/gray eyes yielding a lower match rate (59%) compared to brown (82%) or green/hazel eyes (88%); however, the sample size of blue/gray eyes in the study was not large enough to draw a meaningful conclusion. An isolated case involving an antemortem initial scan collected from an individual on life support yielded an accurate identification (match) with a subsequent scan captured at approximately ten hours postmortem.

Falsely rejected subsequent iris scans or "no match" results occurred in approximately 20% of scans; they were observed at each PMI range and varied from 19%-30%. The false reject rate is too high to reliably establish non-identity when used alone and ideally would be significantly lower prior to implementation in a forensic setting; however, a "no match" could be confirmed using another method. Importantly, the data showed a false match rate of zero, a result consistent with previous iris recognition studies in living individuals.

The preliminary results of this pilot study demonstrate a plausible role for iris recognition in postmortem human identification. Implementation of a universal iris recognition database would benefit the medicolegal death investigation and forensic pathology communities and has potential applications to other situations such as missing persons and human trafficking cases.

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Iris Scanning, Biometrics, Human Identification

H85 A Retrospective Review of All-Terrain Vehicle (ATV) -Related Fatalities in Puerto Rico

Javier G. Serrano, MD, PO Box 361538, San Juan, PR 00936-1538; and Carlos F. Chavez-Arias, MD, PO Box 361538, San Juan, PR 00936*

The goal of this presentation is to describe ATV-related fatalities evaluated during the period from January 2008 to December 2014 at the Puerto Rico Institute of Forensic Sciences.

This presentation will impact the forensic science community by describing 90 ATV-related deaths from a forensic perspective, focusing primarily on the types and mechanisms of injury.

ATVs are three- or four-wheel motorized vehicles intended for use by riders on off-road, non-paved surfaces. ATVs are a popular form of motorized recreation and are also used in a variety of occupational settings including agriculture, construction, and law enforcement. With their rising popularity, there was a corresponding increase in the incidence of injuries and deaths due to ATV accidents.

Ninety cases of ATV-related deaths were received for postmortem examination during the period from January 2008 to December 2014 at the Puerto Rico Institute of Forensic Sciences. Of the 90 cases, 74 (82%) were males and 16 (18%) were females. Three fatalities (3.3%) corresponded to people younger than 16 years of age. The ages ranged from 16 years to 20 years in 14 (15.6%) cases, from 21 years to 30 years in 40 (44.4%) cases, from 31 years to 40 years in 26 (28.8%) cases, and in 7 (7.8%) cases the decedents were older than 40 years. In 71 (79%) cases, the fatally injured person was the driver and in 17 (19%) cases the passenger. Two cases (2.2%) corresponded to a motorcyclist and a pedestrian hit by ATVs. In 75 (83%) cases, the accident occurred on a public paved road and 15 (17%) cases occurred in rural areas. In 89 (99%) of the 90 cases, the ATV was used for recreational purposes and in one case for work-related usage. The mechanism of injury included fall/ejection from the ATV in 67 (74.4%) cases, loss of stability and rollover in 4 (4.4%) cases, and collision with a stationary/moving object in 16 (17.8%) cases. In one case, the ATV and the driver were swept away by a river and in two cases, ATVs hit a pedestrian and a motorcyclist. In 31 (34.4%) cases, the death occurred at the scene, 22 (24.4%) of the injured died the same day under medical care, 27 (30%) died between the 2nd and 5th day of hospitalization, and in 10 (11.1%) cases the death occurred after the 5th day of the hospital stay.

The type of injuries found at autopsy were divided into three groups. Head and neck injuries were present in 85 (94.4%) of the cases, thoraco-abdominal injuries were detected in 51 (56.7%) cases, and severe upper and/or lower extremities injuries occurred in 20 (22.2%) cases. Severe head trauma was the cause of death in 34 (38%) of the cases. In one case, the ATV driver drowned in a river. Blood alcohol level was less than 0.08%/weight in 15 (16.7%) cases and higher than 0.08%/weight in 16 (17.8%) of cases. Among illegal drugs, cannabinoids were detected in eight cases, cocaine in one case, benzoylecgonine in seven cases, and opioids in one case. Alcohol and illegal drugs were detected together in nine cases. In 56 (62%) cases, the toxicology came back negative for both alcohol and illicit drugs. Helmet and other security equipment usage were reported in only one case.

The results of this study show that most of the fatalities involved young male drivers. Passenger fatalities involved mostly females. ATVs lack the general stability of other vehicles and are not meant to be driven on regular paved roads. This study shows that most of the accidents occurred on a public road and during recreational use, which are recognized risk factors for ATV-related deaths. Fall/ejection from the ATV was the predominant mechanism of injury. This mechanism, in addition to the lack of use of a helmet, correlates with the most frequent injury found in this study. Head and neck injuries were present in more than 90% of the cases and severe head trauma was the cause of death in nearly 40% of the cases. Head injuries are frequently fatal in ATV accidents as demonstrated in these cases. Head injuries included subdural and subarachnoid hemorrhages, skull fractures, and brain contusions.

There is a lack of research regarding ATV-related fatalities from a forensic perspective. This study demonstrates a wide spectrum of injuries found at autopsy and correlates them with the mechanisms of injury.

ATV-Related Fatalities, Type of Injury, Mechanism of Injury

H86 Heart Fatty Acid Binding Protein (H-FABP): Early Detection of Myocardial Infarction in Postmortem Analysis

Shashi K. Jasra, PhD, University Windsor, CHS,251 Inter-Faculty Forensic, 401 Sunset Avenue, Windsor, ON N9B 3P4, CANADA; Sean Murphy, BSc, Unversity of Windsor, 401 Sunset Avenue, Windsor, ON N9B3P4, CANADA; Azin Shirin-Bayan, #2 1516 Ontario Street, Windsor, ON N9A4H3, CANADA; Janeta Szczepanik, BS, 4691 Howard Avenue, Windsor, ON N9G 1P8, CANADA; Janelle Hinds, BS, Unversity of Windsor, Windsor N9B3P4, CANADA; Pardeep K. Jasra, PhD, Inter Faculty Programs Forensics, 401 Sunset Avenue, University Windsor, CHS, 151-1, Windsor, ON N9B 3P4, CANADA; and David Shum, MD, Windsor Regional Hospital, 1995 Lens Avenue, Windsor N8W 1L9, CANADA*

The goal of this presentation is to illustrate that with the biomarker H-FABP, early Myocardial Infarction (MI) can be detected.

This presentation will impact the forensic science community by providing a new tool to detect early myocardial infarction with the biomarker H-FABP. This may become a valuable asset for forensic pathologists. This presentation will also provide an opportunity for attendees to explore their ability and performance for the benefit of forensic science.

Postmortem detection of MI is still a challenge for forensic pathologists. Acute MI occurs when the blood supply to the heart is diminished for an extended period of time and the myocardial cellular repair mechanisms fail to maintain homeostasis and re-establish normal cellular functions. In cases where the delay between MI and death is within three to six hours, routine histological microscopic analysis fail to provide enough information since the morphological changes of the damaged tissue are not identifiable. Early detection of MI in postmortem analysis is a valuable asset for forensic pathologists in determining the cause of death.

Following ischemic injury to the muscle tissue of the heart (cardiac tissue), certain chemicals (biomarkers) are released by the damaged cells which can be detected microscopically, utilizing immuno-histochemical techniques. Biomarkers, measurable chemicals released by the damaged cells, are regularly used in diagnosis of MIs in hospitals. This research is a follow-up to a study published in 2012 which analyzed the efficiency of two biomarkers, Troponin-I and Complement C-9, or the early detection of MI.[1]

This study focuses on a new biomarker, H-FABP, in early MI detection. H-FABP is the common name for the gene protein FABP3. This biomarker of interest is a transport protein, oxygenating long chain fatty acids in the cardiac muscle cells located in the myocardium. H-FABP may prove to be a very useful biomarker in early detection of MI with the quick release time of 30 minutes after symptoms have occurred. H-FABP appears to be a very good candidate in early detection of MI in tissue samples, specifically for cases where the conventional Hematoxylin-Eosin (H&E) staining failed to reveal the evidence of such MI. It reveals that MI may be detected within six hours of the onset of symptoms utilizing immune-histochemical staining of the cardiac tissue with H-FABP.

The present study reveals that all the cases in the group where the cause of death was reported as the myocardial infarction at less than six hours showed a negative/remarkably reduced staining whereas the viable myocardium showed a very positive staining for the H-FABP. The biomarker, H-FABP seems to have great potential in detecting the early MI. This early detection of MI will definitely assist in determining the time and cause of death in forensic cases. With no standardized method of determining early MI with routine histology, this research utilizing H-FABP with immuno-histochemical techniques provides a vital asset to pathologists in the near future.

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H-FABP, Myocardial Infarction, Biomarker

H87 Deaths Due to Carbon Monoxide Intoxication Involving Burning Charcoal Briquettes in Enclosed Spaces

Patricia Aronica, MD, OCME, 900 W Baltimore Street, Baltimore, MD 21223; Jack M. Titus, MD, OCME, 900 W Baltimore Street, Baltimore, MD 21223; and David R. Fowler, MD, OCME, 900 W Baltimore Street, Baltimore, MD 21223*

After attending this presentation, attendees will have a complete understanding of carbon monoxide-involved deaths due to the burning of charcoal grills in enclosed spaces.

This presentation will impact the forensic science community by serving as a review of the demographics, toxicology findings, and manner classification of charcoal-related carbon monoxide deaths for comparison to other similar future cases.

Carbon monoxide is an odorless, colorless, and tasteless gas produced from the partial oxidation of carbon-containing compounds when oxygen is insufficient in supply to complete oxidation to carbon dioxide. Sources include cigarette smoke, house fires, and the burning of fuels in cars or trucks, small engines, boats, stoves, lanterns, fireplaces, gas ranges, furnaces, or grills. Charcoal grills use either charcoal briquettes or all-natural lump charcoal as the fuel source. Human exposure to carbon monoxide generally occurs indoors or in semi-enclosed spaces where the oxygen supply may be limited.

A ten-year retrospective search of the electronic database of the Office of the Chief Medical Examiner for the State of Maryland from 2005 to July of 2015 was performed searching for carbon monoxide in the cause-of-death line. Fire-related deaths were excluded. This list was further narrowed to those with grill and/or charcoal listed in the body of the report.

The search revealed 204 cases in which carbon monoxide intoxication or toxicity was listed as the primary cause of death and in which fire-related deaths were excluded. Of these cases, 85 involved vehicle exhaust, 40 involved generators, 17 involved furnaces/hot water heaters, 13 involved lawn mowers, 7 involved space heaters, 5 involved boats, 3 involved gas stoves, 3 involved power tools, 2 involved snow blowers, 4 involved miscellaneous, and 20 involved the burning of a charcoal grill in an enclosed space. In 5 cases, the cause of death was unknown. Of the 20 cases involving charcoal grills, 14 were male and ranged in age from 19 years to 52 years of age with an average age of 39 years. The grills were located in a motor vehicle in 13 cases (65%), in a bathroom in five cases (25%), and 1 case each in a bedroom and an office. Carbon monoxide saturation levels in the cases involving grills ranged from 46% to 81%, overall. Ethanol was positive in 60% of the cases and other drugs were positive in 45% of the cases. Drugs identified were diphenhydramine (three cases), citalopram (three cases), and one case each of venlafaxine, doxylamine, tramadol, and amphetamine. In one case, multiple other drugs were detected. Of the cases in which drugs and/or ethanol were detected, the levels could be considered potentially lethal in three cases. Manner of death was classified as suicide (19) and undetermined (1). Suicide notes and/or e-mails were present in 70% of the cases. No cases of homicide or accident were noted in the grill cases. Body position of the decedent, location in the vehicle/home, charcoal grill type, and position of the charcoal grill varied; however, the most common decedent location was reclined slightly in the driver's seat (7). None of the vehicles or home areas showed extensive internal fire damage. Most of the damage was heat-related and occurred at a point beneath the placement of the grills in the vehicles. Two individuals were found on top of or very close to the grills and showed postmortem thermal injuries on the areas of the body closest to the grills.

In conclusion, carbon monoxide-related deaths involving charcoal grills were not uncommon, representing approximately 10% of non-fire-related deaths in the past ten years in Maryland. The most common enclosed space that the grills were placed was within a motor vehicle. Because enclosed spaces have a limited oxygen supply, this allows for easier production of carbon monoxide by incomplete oxidation of the charcoal to carbon monoxide. In addition, the low and restricted oxygen levels would be associated with little flame damage and a smoldering of the briquettes with very little soot production as the limited oxygen supply would be quickly depleted.

Carbon Monoxide, Charcoal, Grills

H88 Influence of "Final Exit" on Asphyxial Suicides in New Mexico: A Retrospective Review

Adela S. Magallanes, BS, University of New Mexico School of Medicine, 1705 Cagua Drive, NE, Albuquerque, NM 87110; and Hannah A. Kastenbaum, MD*, University of New Mexico, Office of the Medical Investigator, 1 University of NM, MSC 07-4040, Albuquerque, NM 87131*

After attending this presentation, attendees will understand trends of asphyxial suicide in New Mexico utilizing suffocation by plastic bag with or without concurrent inhalation of an inert gas, as suggested by Derek Humphrey's *Final Exit: the Practicalities of Self-Deliverance and Assisted Suicide for the Dying*, originally published in 1991.

This presentation will impact the forensic science community by helping attendees understand trends of asphyxial suicides in their own jurisdictions in regard to probable exposure to the book, "Final Exit," and what characteristics and demographics are common in New Mexico in terms of utilizing the book for a method of suicide.

In 1991, Derek Humphry published *Final Exit: the Practicalities of Self-Deliverance and Assisted Suicide for the Dying* in which he advocated for rational suicide: suicide by individuals who are terminally ill. In the first edition, there were detailed instructions for death by asphyxia by suffocation with a plastic bag over the head with or without premedication with sedatives such as barbiturates and benzodiazepines.[1] A later electronic supplement and second edition published in 2000 updated the recommended pharmaceuticals and suggested the addition of exclusion of oxygen by inhalation of an inert gas like helium. The book is considered controversial as it also advocates for assisted suicide, and populations other than the terminally ill have used its methods.

Suicide, in general, is prevalent in New Mexico and constitutes approximately 7% of all deaths investigated by the state's Office of the Medical Investigator (OMI) annually. This study seeks to evaluate the impact of the publication of Final Exit on suicides in this population. Cases of asphyxial suicide investigated between January 1, 1985, and December 31, 2012, were selected by keyword search in the OMI's electronic database; hangings were excluded. Each autopsy case was evaluated for demographics of the deceased, exposure to Final Exit (evidence of the book at the scene, the presence of an internet search, etc.), presence of any terminal diseases, and history of psychiatric illness. Data were analyzed using logistic regression to determine relationships between independent variables (gender, age, use of inert gas during asphyxiation, etc.) and exposure to Final Exit text.

Of the 122 individuals who died by asphyxial suicide, 45 were female, 77 were male, the vast majority (109) were non-Hispanic Whites, 17 had terminal illnesses, and 52 had a history of psychiatric disease. Age ranged from 18 years to 98 years of age with a mean age of 58. Exposure to Final Exit was determined to be "unknown" in 34 cases, as there was missing investigative information, and these cases were excluded from statistical analysis. Of the remaining 88 individuals, 20 had documented exposure to Final Exit (presence of the text or related materials or an internet search at the scene). Of these 20 individuals, 11 were female, 9 were male, and age ranged from 38 years to 96 old with a mean of 67.9 years. Eight individuals (40%) had a history of psychiatric disease and only four had terminal diseases (20%). Of the 68 individuals without exposure to Final Exit, 21 were female, 47 were male, and age ranged from 18 years to 96 years old with a mean of 56.9 years. Forty-four individuals had a history of psychiatric disease (65%) and four (15%) had a history of terminal illness. The logistic regression model revealed that females committing suicide by asphyxia were more likely to have been exposed to Final Exit and the probability of Final Exit possession increased with age. Males were less likely to have exposure to Final Exit, but likelihood increased with age. Usage of an inert gas increased the probability of Final Exit exposure significantly (53 times).

These results describe the individuals dying of asphyxial suicides and their potential exposure to Final Exit and suggest that its methods are regularly utilized outside Derek Humphry's intended population. Although the annual incidence of suicide by suffocation has risen slightly since 1990, the percentage of deaths investigated by the OMI and ruled to be suicide has stayed constant, averaging 7.4% between 1985 and 2013; however, that exposure to Final Exit could not be determined retrospectively in 34 of the identified cases limits the interpretation of this data. Impact of Final Exit on the incidence of suicidal overdoses, also suggested by the book, was not evaluated and could be a direction for future study.

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Final Exit, Suicide, Suffocation

H89 North Carolina Deaths Involving Acetyl Fentanyl: A Two-Year Retrospective Review

Kimberly E. Janssen, OCME, 3025 Mail Service Center, Raleigh, NC 27699-3025; Justin O. Brower, PhD, CB# 7580, Chapel Hill, NC 27312; Michelle B. Aurelius, MD, NC OCME, 3025 Mail Service Center, Raleigh, NC 27699-3025; and Ruth E. Winecker, PhD, OCME, 3025 Mail Service Center, Raleigh, NC 27699-3025*

After attending this presentation, attendees will have greater insight into the different types of postmortem casework associated with acetyl fentanyl at various concentrations.

This presentation will impact the forensic science community by providing information regarding acetyl fentanyl as it relates to cause and manner of death determinations.

Acetyl fentanyl is a less-active synthetic analogue of fentanyl which is more potent than heroin. Although it has not been approved for use in the United States, clusters of acetyl fentanyl deaths have been reported since 2013. Prior to scheduling by the Drug Enforcement Administration in July 2015, acetyl fentanyl could be ordered over the internet ostensibly as a "research chemical" but in reality is abused in a similar manner to heroin. In the North Carolina Office of the Chief Medical Examiner (NC-OCME) toxicology laboratory, acetyl fentanyl is readily detected in the routine organic bases screen and confirmed and quantitated via a validated Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method. Specimens collected during autopsy generally include blood (both peripheral and central), liver, urine, and vitreous humor. As limited information has been reported on the distribution of acetyl fentanyl in the body, all specimens in cases positive for acetyl fentanyl were submitted for quantitative analysis.

The investigators retrospectively reviewed medicolegal death investigation records, autopsy reports, and toxicology reports at the NC-OCME in order to identify and characterize deaths where toxicology specimens were positive for acetyl fentanyl. In a two-year period, acetyl fentanyl was detected in 18 autopsy cases. Of these, 15 were male (83.3%) and 3 female (16.7%). Twelve were White non-Hispanic (66.7%), four Black (22.2%), one Asian (5.6%), and one American Indian (5.6%). The age of these ranged from 21 years old to 42 years old.

Of the eight finalized reports, acetyl fentanyl was listed as the sole cause of death in 25% of cases, while other drugs or alcohol were contributory in 62.5% of cases. Acetyl fentanyl was detected but not listed as a cause or contributory to death in one case (12.5%). Blood concentrations in these three groups averaged 0.40mg/L, 0.33mg/L, and <0.01mg/L, respectively. Liver concentrations in these three groups averaged 1.6mg/Kg, 1.8mg/Kg, and <0.04mg/Kg, respectively.

Data collected during medicolegal death investigations, including demographics, toxicology results (blood, liver, urine, and vitreous concentrations), autopsy findings, and circumstances (including county and date of death to further characterize a geographic or temporal clustering), will be presented.

Acetyl Fentanyl, Postmortem, Toxicology

H90 Human or Non-Human: Identification of a Gastrointestinal Tract

Sarah Schaerli, Institute of Forensic Medicine, Winterthurerstrasse 190/92, Zurich, SWITZERLAND; Nadja Morf, MS, Institute of Forensic Medicine, Winterthurerstrasse 190/52, Zurich 8057, SWITZERLAND; Michael Thali, MD, Universitat Zurich, IRM/ Forensic Institute, Winterthurstrasse 190/52, Zurich CH-8057, SWITZERLAND; and Dominic Gascho*, Institute of Forensic Medicine Zuerich, Wintherthurerstrasse 190/52, Zuerich 8057, SWITZERLAND*

After attending this presentation, attendees will better understand the different examinations which are necessary to identify soft tissue remains found in the environment and will especially appreciate an easy way to distinguish between human and non-human soft tissue remains.

This presentation will impact the forensic science community by its detailed listing of identification methods referring to the differentiation between human and non-human origin of soft tissue remains.

Introduction: Whenever body remains are found in solitary places, a differentiation between human and non-human material can often be based on visual examination. While bone fragments might be easy to identify visually, soft tissue remains can be rather tricky. Internal organs such as liver, stomach, and bowels were found by a waterguard at the Greifensee in Zurich. A visual examination performed by a veterinary pathologist gave no further insight. Therefore, an identification procedure was implemented based on a Hexagon OBTI test, radiological examinations, and a mitochondrial cytochrome b test.

Materials and Methods: Computed Tomography (CT) scanning was performed on a 128-slice dual source scanner using 120kV with a slice thickness of 0.6mm and an increment of 0.4mm. Reconstructions were made in a soft tissue window with a soft kernel and osseous window with a hard kernel, respectively. Magnetic Resonance Imaging (MRI) was performed on a 3-Tesla magnetic resonance scanner. The performed T2-weighted and T1-weighted inversion recovery sequence parameters are 1.5mm using an XL-torso 16 elements phased-array coil and 2mm using a small-extremity 8 elements phased-array coil with a voxel size of 0.5mm respectively. A common Hexagon OBTI test device was used, which is usually developed for an immunochromatographic rapid test for confirming the presence of fecal occult blood. Mitochondrial cytochrome b test — this mtDNA-based species identification method consists of a multiplex-Polymerase Chain Reaction (PCR) -setup with eight primers varying in their specificity to amplify regions of the mitochondrial cytochrome b gene in different animal classes. After PCR and sequencing, which took approximately two days, the species in origin was identified using Basic Local Alignment Search Tool (BLAST) alignments to the cytochrome b entries in the National Center for Biotechnology Information (NCBI) nucleotide database.

Results: The Hexagon OBTI test showed a negative result for human blood. CT scanning and MRI did not give any specific information. The mitochondrial cytochrome b test showed a match of 99% to silurus glanis, an European wels catfish.

Conclusion: The Hexagon OBTI test devices were originally developed to detect the presence of human hemoglobin as a proof of blood in human stool samples. Because of its human specificity, these test devices are a quick and easy technique to distinguish between human and animal blood and could be used immediately where the remains were found; however, cross-reactions could be shown on samples of primates, mustelidae, and rabbits. Other providers also mentioned that ferret blood may lead to positive results. For a specific identification, the more time-consuming mitochondrial cytochrome b test is a suitable method for a broad spectrum of species.

Identification, Human or Non-Human, Hexagon-Obti-Test

H91 Tire Marks: Don't Tread on Me

Dennis J. Chute, MD, Dutchess County MEO, 168 Washington Street, Poughkeepsie, NY 12601; and Robert J. Bready, MS, Dutchess County MEO, 168 Washington Street, Poughkeepsie, NY 12601*

The goal of this presentation is to examine tire impressions identified on bodies of motor vehicle fatalities.

This presentation will impact the forensic science community by illustrating that tire marks identified on clothing and bodies may assist investigators in the reconstruction of motor vehicle fatalities.

Introduction: Identification of patterned injuries by forensic investigators can be helpful in the reconstruction of the sequence of events in non-natural violent deaths. Although casting tire tread impressions is often described by crime scene investigators, there have been relatively few recent forensic medicine publications of tire marks or tire tread patterns identified on victims of motor vehicle fatalities. Yet a previous report suggested such observations may contribute to one's ability to determine whether a body was erect or lying down at the time of contact with a motor vehicle.[1] Recently, Pircher et al. describes an interesting pattern of blister formation due to tire tread mark.[2] Injuries produced by vehicle tires running over feet have been fairly well described.[3,4] This study seeks to add to the tire pattern literature by describing experiences with roll-over fatalities and tire mark injuries.

Methods: This study reviewed pedestrian versus motor vehicle fatalities in Dutchess County over a ten-year period (2005-2015, n=37) from the records of the Dutchess County Medical Examiner's Office (DCMEO) . All such violent deaths are referred to the MEO per statutes defined by the county's medical examiner code. This study specifically searched for those cases where there was a possibility that a vehicle may have rolled over the victim or contacted the victim during the collision such that the vehicle wheel(s) potentially left a mark on the clothing or body (n=10) . This research did not include cases where it was known that the victim had been struck by one vehicle, then run over by another and almost all of the cases included in this report (nine out of ten) were low-speed encounters. As part of the DCMEO investigation of pedestrian fatalities, police investigators are asked to retrieve for inspection any clothing that may have been worn and/or subsequently removed from the remains in the emergency department or by paramedics during resuscitation attempts. In nine out of ten cases, clothing was inspected as part of the postmortem examination. Photographs taken of the scene, the autopsy, the vehicle wheels, and the undercarriage by police, crime scene technicians, and medicolegal investigators were reviewed.

Results and Discussion: During this period of study, ten cases were identified that suggested the victim may have either gone under the vehicle or come in contact with a wheel based on investigation and/or postmortem findings. Two cases involved a single hit-and-run vehicular manslaughter incident: it was later concluded that one victim was thrown away from the vehicle and did not come in contact with a tire. In six out of remaining nine cases (67%), tire marks were identified on the clothing or body of the deceased. In one of the nine cases, this study concluded that a tire went over the upper forehead/scalp producing an avulsion. Although a tread mark was not identified, such a pattern may have been obscured by the scalp hair. One of the nine cases occurred during winter and heavy clothing, never examined, may have prevented transfer of a tire mark to the body. In six of nine cases (67%), victims went under the vehicle from the side and two victims were run over while the vehicle backed up. In four out of nine (44%) cases, the conclusion was that the pattern identified was a match with the proposed vehicle tire and in the other five (56%) cases that there was not enough detail to reach such a definitive conclusion. Review of the cases suggested the following: (1) fatalities with tire mark transfer typically occur in vehicles (e.g., a truck or tractor) that are higher off the ground than typical passenger/sedan cars (eight out of nine cases or 89%); (2) in low speed roll-over cases, going under from the side of the vehicle is common; (3) tire tread patterns are frequently recognizable but emphasis is placed on recovering articles of clothing in pedestrian versus motor vehicle deaths; and, (4) although tire marks can be identified, this does not mean the pattern found can be definitively matched to the vehicle in question, viz., although supportive, other evidence may be necessary to draw such a conclusion.

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Tire Tread, Tire Mark, Pedestrian

H92 Micro-Computed Tomography (CT) Analysis of Deadly Gunshot Wounds

Paolo Fais, MD, Section of Legal Medicine - Universitiy of Verona, GB Rossi Hospital, P.le LA Scuro 10, Verona 37134, ITALY; Chiara Giraudo, MD, Via giustiniani 2, Padova , ITALY; Guido Pelletti, MD, University-Hospital of Padova, Section of Legal Medicine, via Falloppio 50, Padova 35131, ITALY; Alessia Viero, MD, Legal Medicine and Toxicology Unit, via Falloppio 50, Padova 35121, ITALY; Diego Miotto, Radiology Section, Dept of Medicine, Univers, Via Giustiniani 3, Padova, AA 35121, ITALY; Massimo Montisci, PhD, Via Falloppio 50, Padova, ITALY; and Giovanni Cecchetto, MD, PhD, Legal Medicine and Toxicology Unit, Via Falloppio 50, Padova 35121, ITALY*

After attending this presentation, attendees will be aware of the advantages and the limitations related to the application of micro-CT for the analysis of gunshot wounds.

This presentation will impact the forensic science community by demonstrating the importance of performing radiological postmortem investigations, including micro-CT analysis of firearm wounds, for reconstructing the shooting incident.

Radiological techniques for the study of gunshot wounds can be used to detect foreign bodies such as metallic fragments or projectiles, document the bullet path, discriminate osseous entry and exit wounds, and evaluate any associated tissue damage prior to autopsy.[1-5]

This study tested micro-CT (a radiological technique which provides greater spatial resolution with respect to clinical computed tomography) for the examination of gunshot wounds experimentally produced on human skin (fresh, decomposed, charred, submerged, and covered by textile specimens) in order to perform tridimensional reconstructions of the spatial distribution of Gunshot Residue (GSR) particles.[6-8]

The results of this experimental study demonstrated that micro-CT analysis is an objective and rapid tool for estimating the firing range in intermediate-range gunshot wounds as well as for differentiating Entry Wounds (EntW) from Exit Wounds (ExtW).

Presented is a case series of seven gunshot-related deaths (two dyadic deaths, two suicides, and one homicide). For each case, a comprehensive crime scene investigation, including criminalistics analysis, was performed. On each corpse, an unenhanced Multi-Slice Computed Tomography (MSCT) was performed before the autopsy. During the forensic autopsy, gunshot wounds were sampled (i.e., skin specimens comprising the substance defect and the surrounding epidermis, dermis, and subcutaneous fat) for micro-CT and histological analysis.

Twelve firearm wounds were analyzed. Eight were located on the head/skull (five EntW and three ExtW), and four were located on the thorax (two EntW and two ExtW). Micro-CT analysis evidenced radiopaque particles in all the entry lesions, providing information concerning the firing range and the differential diagnosis between the EntW and ExtW.

Hyperdense particles were also detected on the three exit lesions located on the head. The morphological evaluation of that material (cubic-shaped fragments and smaller roundish particles) suggested the presence of at least two kinds of material: metal GSR-like particles and bone fragments, which were indistinguishable based on the radiological density.

In all cases, the integration of radiological, autopsy, and histological data allowed the reconstruction of the trajectory of the gunshot and the most probable dynamics of the event.

The most important limit of micro-CT analysis is that this technique allows only a presumptive identification of the GSR particles and, consequently, substances with the same density of powder particles might be erroneously tagged as GSR, generating a false positive result. In particular, further studies are required to solve bone contamination problems whether on a radiological basis (i.e., low voltage micro-CT protocols, multiple thresholds, etc.) or on a chemical basis (i.e., decalcification of the specimen without altering its GSR content).

Considering that micro-CT is a non-destructive technique, a possible solution for the above-mentioned problem could be the identification of the chemical composition of the radiopaque residues by means of an environmental scanning electron microscope coupled to an X-ray fluorescence energy dispersive spectroscope.

In conclusion, micro-CT analysis may furnish important information on the trajectory of the gunshot and the dynamics of the event and, in combination with other autopsy and crime scene findings, may play an important role in reconstructing the shooting incident.

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Micro-CT, Gunshot Wounds, Gunshot Residues

H93 When Insect Colonization Starts Before Death: A Case From Central Italy

Stefano Vanin, PhD, Queensgate, Huddersfield HD1 3DH, UNITED KINGDOM; Martina Focardi*, Largo Brambilla 3, Florence 50134, ITALY; Manuela Bonizzoli, MD, Neuromusculoskeletal and Sensory Organs Dept, Largo Brambilla 3, Florence 50134, ITALY; Marialuisa Migliaccio, MD, Neuromusculoskeletal and Sensory Organs Dept, Largo Brambilla 3, Florence, ITALY; Laura Tadini Buoninsegni, MD, Neuromusculoskeletal and Sensory Organs Dept, Largo Brambilla 3, Florence, ITALY; Marco Mangini, MD, Neuromusculoskeletal and Sensory Organs Dept, Largo Brambilla 3, Florence, ITALY; and Vilma Pinchi, PhD, via Della Resistenza 14, Murlo, Siena 53016, ITALY; Gian A. Norelli, sez.dep.Medicina Legale, Firenze, ITALY*

After attending this presentation, attendees will better understand the case of a woman with a severe myasis found unconscious in her garden five days after having last been seen alive. Attendees will realize that in cases such as this, in case of death, an entomological evaluation of the time since death would be completely wrong.

This presentation will impact the forensic science community by demonstrating that flies can colonize unconscious bodies before death and in so doing can affect the minimum Postmortem Interval (mPMI) estimation based on the entomological approach.

Forensic entomology is a branch of forensic science in which insects are used as evidence in legal investigations relating to humans or wildlife. The examination, identification, and analysis of insects associated with human remains, combined with the knowledge of insect biology, can provide a further level of detail in addition to medical and anthropological data in the reconstruction of events occurring close to the time of death. In particular, necrophagous insects are useful in studying Postmortem Interval (PMI), postmortem transfer, and presence of drugs or poisons. One of the theoretical pillars on which the discipline is based concerns the fact that flies colonize a body after death; however, in cases of myases (infestation of parasitic fly larvae on living or necrotic tissues in living vertebrates), maggots are present before the death of the individual with consequences in the correct estimation of the mPMI if based only on the entomological approach.

In this presentation, a case is reported of a woman who was found alive lying in her garden for four days before being rescued. The living woman was largely colonized by fly larvae.

In July 2015, an 84-year-old woman, affected by arterial hypertension and hyperthyroidism, was found unconscious by the emergency rescue team in the garden of her home. The pre-hospital evaluation found the patient hypertensive, hypoglycemic, with a severe infestation of fly larvae on the face and particularly on the conjunctivae, the nasal choanae, the mouth, and the external auricular ducts. A sore was detected in the sacral region, potentially related with her lying position. The patient was also affected by first- and second-degree sun burns on the exposed parts of her body: the abdomen and part of the thorax.

A total body Computed Tomography (CT) scan of the woman showed a left cerebral intraparenchimal capsulo lenticular hemorrhage. The larvae infestation was reported in the bronchi, rectum, vagina, and the external auricular ducts with bilateral multiple perforations of the tympanic membranes. Larvae, on the second and third instars were collected and identified as members of the *Calliphoridae* family. A second CT scan reported a bilateral purulent ethmoidal, maxillary sinusitis, that received surgical drainage. No larvae were found in the affected sinuses. The infestation evolved in the perforation of bilateral tympanic membranes, maxillary sinusitis, and bacterial pneumonia. The sacral wound was treated with escharectomy and debridement of purulent material

The woman had been observed being active five days before being rescued and died the day after the rescue. In this case, a classic entomological evaluation would have provided a completely erroneous mPMI. This case is an example of how unconscious bodies can be colonized before death and how it can affect the time-since-death evaluation if based only on the entomological approach. In suspicious conditions, histological analysis of the colonized tissues can be helpful in order to evaluate if colonization started before or after death of the victim.

Entomological Evaluation, PMI Estimation, Myasis

H94 Medicolegal Issues in Lethal Necrotizing Fasciitis: Presentation of a Case Series

Paolo Fais, MD, Section of Legal Medicine - Universitiy of Verona, GB Rossi Hospital, P.le LA Scuro 10, Verona 37134, ITALY; Giovanni Cecchetto, MD, PhD, Legal Medicine and Toxicology Unit, Via Falloppio 50, Padova 35121, ITALY; Renzo Giordano, MD, Dolo Hospital - Unit of Pathology, Via XXIX Aprile 2, Dolo (VE), CA 30031, ITALY; Massimo Montisci, PhD, Via Falloppio 50, Padova, ITALY; Dario Raniero, PhD, University of Verona, Dept of Public Health, Ple LA Scuro 10, Verona, ITALY; Federica Bortolotti, PhD, MD, Dept Medicine & Public Health, Policlinico GB Rossi, P. le Scuro No. 10, Verona 37134, ITALY; and Franco Tagliaro, PhD, MD, Dept of Medicine & Public Health, Policlinico GB Rossi, P. le Scuro No. 10, Verona 37134, ITALY*

After attending this presentation, attendees will better understand the clinical and postmortem features of Necrotizing Fasciitis (NF) and its implications on medical malpractice and liability.

This presentation will impact the forensic science community by providing a broad and thorough review of the current literature and scientific knowledge about NF, with emphasis on key issues of interest to the medicolegal investigator and forensic pathologist.

NF is a rare but severe infection of the soft tissues, usually related to a bacterial invasion of the fascia, quickly spreading to muscles, subcutaneous fat, and to the overlying skin. The forensic pathologist often encounters NF in cases of alleged clinical malpractice because the infection usually leads to a rapid death in apparently healthy subjects with no obvious clinical diagnosis.

Here a series of five cases (aged 30 years—50 years old) involving death due to NF are presented. In all of the reported cases, patients presented to the Emergency Room (ER) with symptoms of pain, sometimes associated with mild fever (three cases). In three cases, a previous trauma was reported in the anamnesis. Predisposing factors for NF, such as myelodysplastic syndrome, obesity, and liver cirrhosis, were observed in only two cases. Imaging studies (plain radiographies in three cases and Computed Tomography (CT) scan in two cases) were performed on the areas affected by pain and revealed a thickening of soft tissues only in two cases. Two cases were discharged after their first medical consult. In all of the reported cases, signs of local phlogosis, such as hemorrhagic suffusion of soft tissues, lesions on the skin, and bullae were absent at admission in the ER and appeared only later and concurrently to the diagnosis of septic shock. When systemic involvement was observed, basic blood tests such as basic biochemical analyses, complete blood count, and, in two cases, C-reactive protein tests were performed. In only one case was an antemortem clinical diagnosis of NF reached.

In all the reported cases, a forensic autopsy was performed. A necroscopic examination was performed, detecting in all of the cases reported signs of local phlogosis, such as violaceus, swollen and tender skin with crepitus at external inspection, and signs of necrosis, such as grayish-colored fascia and diffuse hemorrhagic infiltration of the muscular tissue during dissection. The histopathologic examination of such tissues displayed necrosis, diffused angiothrombosis with interstitial infiltration of granulocytes, and intramuscular colonies of bacteria. Microbiological analysis detected *Streptococcus pyogenes* in three cases, *Staphylococcus aureus* in one case, and *Staphylococcus aureus* plus *Escherichia coli* in one case.

More than half of patients developing NF have a pre-existing medical condition and the portal of entry of microorganisms is usually a post-traumatic discontinuation of the skin and the soft tissues above the fascia; however, as observed in this case series, sometimes NF occurs in apparently healthy subjects who have no previous history of trauma.[1-4]

A diagnosis of NF may take advantage of a detailed clinical evaluation, laboratory diagnostic parameters, and radiological investigations.[5-9] An early diagnosis of NF allows for a timely surgical therapy, which is usually the strongest predictor for a better patient outcome; however, a prompt clinical diagnosis of NF is usually difficult or even unfeasible because of the non-specificity of symptoms at the time of presentation.[10]

In the case series discussed, the presenting symptoms were vague and more specific signs of NF (hemorrhagic suffusion of the skin) appeared later and concurrently to septic shock, leading to a rapid death. In the absence of a clinical diagnosis, radiological data and laboratory diagnostic tools were not requested by the physicians. Moreover, in one case, the diagnosis of NF was made only through emergent surgical exploration. On these grounds, the most important diagnostic issue remains a high index of clinical suspicion which is essential for the formulation of a timely clinical suspect of NF; however, this can be a complex and difficult task because presentation symptoms are extremely vague and NF is infrequent (usually practitioners encounter one or two cases in their entire careers).[11]

Even if a prompt and correct therapy (broad spectrum antibiotic therapy, aggressive surgical debridement of necrotic tissue with limb amputations) is performed in a timely manner, NF cases may pose medical malpractice disputes because of its possible fatal outcome in otherwise young and healthy people.

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Forensic Pathology, Necrotizing Fasciitis, Medical Malpractice

H95 Recreational Sporting Activity Vehicle-Related Deaths

Samuel Prahlow, Valparaiso University, 1212 Galien-Buchanan Road, Galien, MI 49113; Andrew Renner, MD, Indiana University School of Medicine, Dept of Anesthesia, Indianapolis, IN 46202; Abigail J. Grande, BS, WMU Homer Stryker MD School of Medicine, 1000 Oakland Drive, Kalamazoo, MI 49008; Joyce L. deJong, DO, WMU Homer Stryker MD, School of Medicine, Dept of Pathology, 1000 Oakland Drive, Kalamazoo, MI 49008; and Joseph A. Prahlow, MD, Western Michigan University School of Medicine, 300 Portage Street, Kalamazoo, MI 49007*

After attending this presentation, attendees will: (1) understand the diversity of case types involving vehicle-related deaths that occur during recreational sporting activities; (2) become familiar with some of the risk factors associated with such deaths; and, (3) recognize strategies that may be employed to reduce the risk of such deaths.

This presentation will impact the forensic science community by focusing attention on a somewhat unique yet much-too-common category of accidental death. By examining these cases, the forensic science community can be instrumental in advocating for implementation of preventive strategies, which may reduce the number of deaths resulting from vehicle use in recreational sporting activity settings.

Accidental deaths remain a major public health concern within the United States and elsewhere throughout the world. Unintentional motor vehicle/traffic incidents rank among the top three leading causes of injury-related deaths in all age groups within the United States.[1] A less common subgroup of vehicle-related fatalities occur outside of the setting of typical "traffic" conditions.[1] Another less-common category of death involves sports- and recreation-related deaths. These include deaths related to underlying natural disease and precipitated by exertion, those related to trauma, and those related to an adverse environment.[2] If one eliminates deaths related to competitive sports from consideration, traumatic sports- and recreation-related deaths in which a vehicle of some type is involved represent a relatively small, but not insignificant, group of diverse case types. For the purposes of this study, a "vehicle" is considered a means of carrying or transporting something, with the "something" representing the participant in the sporting/recreational activity.[3] The study excludes flying vehicles and non-mechanical modes of transport, such as horseback. This study examines a series of cases in which death occurred in the setting of a non-competitive recreational sport activity utilizing a mechanical vehicle. The study includes motorized and non-motorized vehicles, land and water vehicles, and summer and winter recreational activities.

The series of cases come from the collective experience of several personnel and includes cases from several different jurisdictions. The series does not include every case that fits the criteria for this study and it is not meant to be all-inclusive. Instead, the cases were purposefully selected in order to provide a sampling of the wide range of case types that exist. Cases presented include deaths related to motorized vehicle use on land in warm weather, including go-carts and All-Terrain Vehicles (ATVs); deaths related to non-motorized vehicle use on land during warm weather, including bicycles; deaths occurring during the use of water-based vehicles during warm weather, such as Personal Watercraft (PWC) and boats; deaths related to motorized vehicle use during winter weather, including snow machines (snowmobiles); and deaths due to non-motorized vehicle use during winter weather, such as sleds and skis. The details of the cases are presented, with special emphasis placed on potentially modifiable risk factors which exist in each case.

Since these cases tend to occur in situations where participants are purposefully attempting to enjoy themselves, often with a history that the activity has been performed numerous times previously without incident, the unanticipated lethal outcome is particularly shocking to witnesses/other participants. As with many accidental traumatic events, deaths related to vehicle use in recreational sporting activities may be related to a variety of risk factors, including but not limited to: the presence of dangerous environment/surroundings; disrepair, inappropriate modification of, or other malfunction of the vehicle; improper use of, failure to use, or ignorance of appropriate safety equipment/devices; operating the vehicle while under the influence of alcohol or drugs; inexperience on the part of the vehicle operator/participant; not using the vehicle in the appropriate manner, including performing dangerous maneuvers and/or exceeding recommended speeds; and the willingness of the participant to engage in reckless, dangerous behavior.

Because of the speed and forces involved in certain land- and water-based, vehicle-related recreational activities, persons who participate in these sporting activities are at-risk for injury and death. By identifying and understanding the factors that contribute to an increased risk for injury and death in these activities, forensic scientists can help to formulate guidelines for safer participation. When abiding by safety precautions and suggested guidelines for appropriate participation, persons engaging in land- and water-based recreational sporting activities that involve the use of vehicles will be better able to prevent catastrophic injury and death.

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Accidental Death, Recreational Sports, Vehicle-Related Death

H96 Using Microbial Communities to Estimate the Postmortem Interval: A Validation Study

Courtney Weatherbee, BS, Michigan State University, 243 Natural Science, 288 Farm Lane, East Lansing, MI 48824; Jennifer L. Pechal, PhD, Michigan State University, 243 Natural Science Bldg, East Lansing, MI 48824; Trevor I. Stamper, PhD, Purdue University, Dept of Entomology, 901 W State Street, West Lafayette, IN 47907; and M. Eric Benbow, PhD, Michigan State University, Depts of Entomology & Osteopathic Med Specialties, 288 Farm Lane, East Lansing, MI 48824*

After attending this presentation, attendees will understand the potential of using microbial community succession to estimate the minimum Postmortem Interval (PMI_{min}). Estimating a PMI_{min} range is an important forensic tool that can assist investigators in establishing the timeline of when a person died and in identifying potential suspects. The PMI_{min} is a significant element of solving crimes, yet is a difficult piece of information to estimate or predict using existing, and often fragmented, evidence collected during a death scene investigation. Current methods employed to narrow the estimated time of death include rigor mortis and patterns of insect succession and development; however, recent advances in molecular technology now offer the possibility of utilizing the postmortem activity of microorganisms, such as bacteria and archaea, in estimating the PMI_{min}.[1]

This presentation will impact the forensic science community by offering validation of a novel approach to forensic microbiology. In one of the first studies to explore the potential use of microbial communities and next generation technology in estimating the PMI_{min}, Pechal et al. used swine carcasses as surrogates for human cadavers and high-throughput metagenomic sequencing to characterize the microbial community profiles of the buccal cavity and skin throughout decomposition.[1] Their results determined that there was no statistically significant difference among the replicate carcasses, but there were statistically significant differences among days and sampling regions (buccal vs. skin). By calculating the taxon richness and relative abundance at the phylum and family level for each time point, they were able to identify indicator taxa and develop models for estimating the PMI_{min} using Indicator Species Analysis (ISA) and random forest analysis. Indicator phyla identified were Bacteroidetes, Proteobacteria, Actinobacteria, and Firmicutes. At both the phylum and family level, there was a negative linear relationship of taxon richness over time. Overall, the most accurate model was produced using ISA at the family level with 94.4% of time since placement of remains explained by the postmortem microbial communities. Utilizing this microbial succession information as a forensic tool will likely enable investigators to devise additional methods for collecting evidence and improve accuracy of estimating the PMI_{min}; however, the next step in developing this approach as a new microbiological tool is to perform validation studies and estimate error in PMI_{min} estimates.

To validate the microbial succession models of Pechal et al. and better understand community succession at the microscopic level throughout decomposition, a replicate survey study was conducted using six swine (*Sus scrofa* L.) carcasses in an open field surrounded by forest in Indiana. Individual epinecrotic microbial community samples were aseptically collected using sterile cotton-tipped swabs from three regions on each carcass: the buccal cavity, a transect of abdominal skin, and the anal region. Sampling occurred every 12 hours at 7:00 a.m. and 7:00 p.m. for eight days, which was the amount of time for the carcasses to fully decompose and blow fly (Diptera: Calliphoridae) larvae to migrate from the resource to pupate. All samples were stored at -20oC until the microbial DNA could be isolated using a commercially available DNA extraction kit. Using 16S amplicon metagenomic sequencing, the microbial communities were characterized (taxon identification and relative abundance) over decomposition time. Once community profiles were determined, the relative abundance and taxon richness trends were compared to the findings of Pechal et al. The indicator phyla previously identified (Bacteroidetes, Proteobacteria, Actinobacteria, and Firmicutes) were also the most predominant taxa in this replication study, and analysis of the relative abundance over time revealed additional similar trends between studies. Taxon richness also decreased over decomposition at both the phylum and family level for this study.

The concept of postmortem microbial succession as a forensic tool has great potential, but has only recently been realized and made possible by next generation technology, which is why a study like this is so critical. By validating proposed microbial succession models, this study provides an important step toward advancing the field of forensic microbiology and increasing accuracy of estimating the PMI_{min}.

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Microbial Succession, Postmortem Interval, Decomposition

H97 Forensic Botany: Judicial or Circumstantial Evidence? A Case Report and Review of the Literature

Isabella Aquila, MD, Viale Europa, località Germaneto, Policlinico Universitario, S Venuta-Medicina Legale, Catanzaro 88100, ITALY; Ciro Di Nunzio, MFS, PhD*, Magna Graecia University, Viale Europa, Germaneto, Legal Medicine, Catanzaro 88100, ITALY; Silvia Boca, Viale Europa, Catanzaro, ITALY; and Pietrantonio Ricci*, Viale Europa-Località Germaneto, Catanzaro, ITALY*

After attending this presentation, attendees will understand the impact of forensic botany on crime scene investigation.

This presentation will impact the forensic science community by demonstrating the importance of judicial evidence that comes from the use of forensic botany.

Forensic botany is a discipline which uses the knowledge of botany as an aid in solving the crime. The important presence of plant species can be very useful in forensics as plant science can be used as evidence in court. Forensic science encompasses many subdisciplines. It can be subdivided into several botanical subspecialties, including plant anatomy (the study of cellular features), plant systematics (taxonomy and species identification), palynology (the study of pollen), plant ecology (plant succession patterns), and limnology (the study of freshwater ecology). Plant species identification can determine the geographical origins of a sample, provide links between a crime scene and suspects, and test alibis. Plant evidence can be useful to determine if a death is due to an accident, suicide, or homicide or in determining what time of year a burial may have taken place.

Case Report: This forensic case illustrates the importance of botanical evidence that specifically had been collected from clothes of the victim who was found dead in dubious circumstances. This report is the case of a man of Romanian nationality who arrived at the hospital in serious medical condition due to electrical injuries and died a few hours after his arrival. His wife reported that he was repairing a chandelier in the house when he was electrocuted, fainted, and consequently fell from a ladder. Investigators and a forensic pathologist did not believe this version of events and performed an inspection of the house and an autopsy. During the inspection, a ladder with rubble and blood stains on the ground were discovered. This blood was analyzed. Consequently, the autopsy showed the presence of small vegetal elements on the head and clothes of the subject. These vegetal elements were identified as *Xanthium spinosum*, an herbaceous plant belonging to the family *Asteraceae*, with characteristic spiny fruit. The body presented with severe burns and areas of skin necrosis on the arms, trunk, and left leg. Furthermore, a head injury with areas of brain hemorrhage and fractures of the skull base were identified. The histological examination revealed that the passage of electricity had generated dermal necrosis. Therefore, the size of the lesions detected were not compatible with an electric shock by low voltage. Even with the results of the autopsy regarding the cause of death, the manner of death, and the circumstances in which it had occurred, the case remained unsolved. As a result, a survey was performed on the botanical elements detected during the external examination of the body. The botany survey analyzed the nature of the plant matter found on the clothing. The typical habitat of *Xanthium spinosum* is the uncultivated and arid ruderal areas. The favorite substrate is both calcareous and siliceous with pH neutral and high nutritional values of the soil which should be dry. In many areas, it is considered an invasive weed. In the mountains, these plants can be found up to 1,000m, but their presence is especially noticeable on hilly areas, plains, and plains at sea level. An analysis of the origin of these botanicals was connected to a specific geographical area of the place where the investigators focused their survey to get more results. The limits of the geographical area allowed for the clarification of the dynamics of events. Investigators identified the trellis of a high-voltage powerline on which the man, trying to steal copper from electrical cables, was electrocuted and thrown to the ground by the shock. When he fell, the botanical elements attached to his clothes and helped investigators distinguish between the primary real crime scene and the secondary fictitious scene developed by relatives (who were later arrested) in order to conceal the theft of copper.

Conclusions: In conclusion, crime scene evaluation is necessary to preserve and collect the features of the crime scene in order to show the relationship of that evidence to the overall scene and to other evidence.

Forensic Science, Forensic Botany, Evidence

H98 The Forensic Applications of 3D Postmortem Multislice Computed Tomography (PMCT): From "Radiopsy" to "Virtopsy"

Isabella Aquila, MD, Viale Europa, località Germaneto, Policlinico Universitario, S Venuta-Medicina Legale, Catanzaro 88100, ITALY; Ciro Di Nunzio, MFS, PhD, Magna Graecia University, Viale Europa, Germaneto, Legal Medicine, Catanzaro 88100, ITALY; Carmela Falcone, MD, Viale Europa, Policlinico S. Venuta, 88100, Catanzaro 88100, ITALY; Oscar Tamburrini, PhD, Viale Europa, Germaneto 88100, Catanzaro 88100, ITALY; Silvia Boca, Viale Europa, Catanzaro, ITALY; and Pietrantonio Ricci*, Viale Europa-Località Germaneto, Catanzaro, ITALY*

After attending this presentation, attendees will better understand the ability of virtual autopsy to make qualitative improvements in forensic pathology.

This presentation will impact the forensic science community by demonstrating the applicability of 3D PMCT in obtaining repeatable and reproducible results in radiological investigations.

The autopsy represents the most appropriate investigative tool in the analysis of the cause of death. In spite of this, the role of diagnostic imaging in forensics has also become increasingly important. The documentation and analysis of postmortem findings, obtained with diagnostic imaging, are operator-dependent, objective, non-invasive, and will lead to qualitative improvements in forensic investigations. Various studies have demonstrated the applicability of radiology in forensics. Radiological methods, when applied for forensic purposes, form the so-called virtual autopsy or virtopsy. Currently, PMCT, which is carried out prior to the autopsy, is considered a valuable aid in forensic investigations. PMCT has become a useful tool in various fields of forensic pathology. It allows the acquisition of data that, together with the autopsy findings, make it possible to answer different medicolegal questions in a more accurate and precise way.

The goal of this study was to demonstrate the applicability of this method in order to obtain repeatable and reproducible results. To attain this goal, 20 different cases of forensic interest were studied. These cases were divided into five groups (Group A through Group E) according to the cause of death. Group A consisted of three bodies whose cause of death was attributed to asphyxia; in group B, eight cases of gunshot wounds were studied; in group C, four exhumed corpses and human remains were analyzed; in group D, three cases of traumatic death were studied; and, in group E, two cases without a specific cause of death were studied.

For each corpse, a total-body Multi-Slice Computed Tomography (MSCT) with a 64-slice MSCT system supplemented with 3D reconstructions of the entire skeletal system was performed. After the CT scan, a standard autopsy was executed, including both external and internal examinations. Next, the analysis and comparison of postmortem radiological data with the autopsy data was completed. In this study, the focus was on specific targets: hollow organs, parenchymal organs, the skeleton, blood vessels, soft tissues, and blood effusion in natural cavities. In the case of human remains, an anthropological study consisting of anthropometric analysis was performed. Additional benefits of using this method include the ability to provide clear and accurate information that can be submitted for judgment to the court as forensic evidence, the documentation of investigation through a 3D reconstruction, and a better quality of documentation due to the storage and transfer of digital data.

In this study, PMCT proved to be very useful in the reporting of alterations of the facial area, with particular emphasis on the masticatory system, in the evaluation of the presence of a tracheal foreign body, in the identification of airway obstruction, in the identification of skeletal injuries and parenchymal lesions, and in the visualization of perilesional gas bubbles; however, the applicability of this method has shown some limitations regarding the identification of small vessel lesions, the visualization of vascular thromboembolism, the identification of infiltration of the soft tissues, and the identification of fractures of the cranial base. In particular, it was discovered that an important disadvantage of the method consisted of an overestimation of hemorrhagic effusion in natural cavities.

Conclusions: According to the literature, one can conclude that virtual autopsy today represents a valuable support to the investigation of forensic pathology but it cannot be considered as an alternative to the usual postmortem procedures. This virtual approach is not invasive or even minimally invasive and together with the documentation and analysis of postmortem findings, it will allow qualitative improvements in forensic medicine in the near future.

Forensic Sciences, Virtopsy, Forensic Pathology

H99 Right Atrial Infarction With Rupture

Sait Özsoy, MD, Gulhane Military Medical Academy, School of Medicine, Dept of Forensic Medicine, Etlik-Kecioren, Ankara 06018, TURKEY; Sultan Pehlivan, Ankara Branch of the Council of Forensic Medicine, Ankara 06300, TURKEY; Bahadir Özen, Sivas Adli Tip Sube Müdürlügü, Sivas, Türkiye, TURKEY; and Gulnaz T. Javan, PhD, Alabama State University, Forensic Science Program, 915 S Jackson Street, Montgomery, AL 36104*

After attending this presentation, attendees will better understand rarely seen acute atrial infarction cases.

This presentation will impact the forensic science community by providing information concerning the importance of examination of the atrium in cardiac death cases.

Cardiac death usually occurs as a result of severe atherosclerosis of coronary arteries dependent left ventricular myocardial infarction induced ischemic heart disease; however, infarction can occur outside of the ventricles and in the other parts of the heart. Atrial infarct is a rare condition in ischemic heart disease. It is characterized by insidious advancement and non-specific electrocardiographic findings that make its diagnosis prior to death a rare occurrence.

This presentation discusses the case of a 33-year-old male victim with a rare case of non-atherosclerotic, unruptured, aortic intima separated, disruption of coronary blood supply dependent atrial infarction.

The case of a 33-year-old male with moderate mental retardation (IQ=40) with right atrial infarction dependent rupture of the transmural is presented. On a winter day, the man felt ill on the street and, following Cardiopulmonary Resuscitation (CPR) by first responders, died in the ambulance while being transported to the hospital. An autopsy was performed due to the sudden, unexpected death. On external examination, there was no sign of trauma to the chest or to either of his arms beyond CPR-related injuries. A CPR-related broken sternum and bruises were found. There was no gross pathology in the trachea or lungs. Six hundred milliliters of clotted fluid was emptied from the pericardial sac. The heart weighed 390 grams. A rupture measuring 4cm x 3cm was detected in the rear wall of the right atrium of the heart. Heart valve circumference measurements were within normal limits. Thickness of the right ventricular wall was measured at 0.7cm and the left ventricular wall at 1cm. Aortic dissection was found in the first 2.5cm-3cm of the aorta which was the cause of the bleeding found in the region. The lumen of the coronary artery was found to be clear.

In the histopathological examination, the area neighboring the right atrium rupture section showed early signs of fibroblastic activity consistent with three-day to seven-day-old Myocardial Ischemic (MI) findings. Myocardial fibers had signs of bleeding which is a characteristic of 4-12 hour post-MI. In sections of the vessel wall of the aorta, signs of bleeding and segregation in the media layer, foci of mononuclear inflammatory cells in adventitia, and hemorrhage foci were detected.

Toxicological examination revealed the presence of chlorpheniramine, ephedrine, pseudoephedrine, phenylpropanolamine, acetaminophen, and naproxen. The quantity of these ingredients was not at a toxic level. After examination of all toxicological, histopathological, and autopsy findings, a conclusion of “acute right atrium infarction dependent atrium rupture and cardiac tamponade” as cause of death was made. Despite the openness of the coroner artery lumen, separation of the ascending intimal aorta caused narrowing of the right aortic ostia and therefore resulted in an infarct in the right atrium.

Atrial Infarction, Atrial Rupture, Autopsy

H100 Lung Weights in Deaths Due to Drug Intoxication

Heather I. Chen, BA, Western Michigan University SOM, 300 Portage Street, Kalamazoo, MI 49007; and Joyce L. deJong, DO, WMU Homer Stryker MD, School of Medicine, Dept of Pathology, 1000 Oakland Drive, Kalamazoo, MI 49008*

After attending this presentation, attendees will better understand that a finding in deaths due to drug intoxication is increased lung weights. In the course of comprehensive death investigations, lung weights are valuable to determine if the death was due to the toxic effects of drugs. Lung weights are not valuable in determining the manner of death. In addition, this presentation will illustrate how certain factors such as age, presence of pneumonia, and resuscitation attempts affect lung weights.

This presentation will impact the forensic science community by providing additional indicators of death to consider in the investigation of deaths possibly due to drug intoxication.

A retrospective study of autopsy data to determine whether increased weights of the lungs can be considered a reliable supporter of drug overdose as the cause of death was conducted. In previous literature, normal lung weight at autopsy for men has been determined to be about 445g for the right lung and 395g for the left lung (840g total).[1] The lung weights for women are slightly lower at 340g for the right lung and 299g for the left lung.[2]

This retrospective study analyzed data from individuals whose deaths were caused by the toxic effects of drugs. The deaths occurred in 2014 in Michigan and totaled 133 deaths. The manners of death were classified as accidents (109 cases), suicides (15 cases), and indeterminate causes (9 cases). Resuscitation attempts were made on 27 of the individuals in the study and 16 individuals were found to have acute pneumonia (bronchopneumonia or lobar) on autopsy.

The average lung weight among cases examined were 700g for the right lung and 613g for the left lung (1,313g total). When compared to previous reports of normal lung weight, the lung weight of people who had died of a drug overdose is significantly higher. In addition, other factors that might contribute to the increase in lung weight and also support a diagnosis of death due to the toxic effects of drugs were identified. The average weight of lungs in which pneumonia was identified was greater than the average weight of lungs in the study population without pneumonia with 95% confidence in all lung weights for both gender groups. The same level of confidence (95%) was found in the lung weight of people who were resuscitated as compared to those who were not resuscitated. It should be noted that there was no statistical significance in lung weight between accidental deaths compared to deaths determined to be a suicide or indeterminate.

In conclusion, the nearly two-fold increase in average lung weight in these subjects compared to normal lung weight as reported in the literature suggests that drug overdose can be a cause of increased weights of the lungs. Conversely, an increased lung weight may be supportive of drugs causing the death as opposed to other competing factors. In addition, factors such as evidence of pneumonia and attempted resuscitation are associated with increased lung weight. An increased lung weight can be used as an indicator of death due to drug overdose, granted the presence of other pieces of evidence such as a history of drug abuse, toxicology levels, and scene paraphernalia.

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Lung Weight, Drug, Intoxication

H101 Heroin and Asthma Deaths in Cook County, Illinois — A Two-Year Review

Serenella Serinelli, MD, Sapienza University, Dept of Anatomy, Histology, Forensic Medicine, & Orthopedics, Viale Regina Elena 336, Rome, Lazio 00169, ITALY; Matthew F. Fox, MD, Rush University Medical Center, 1653 W Congress Parkway, Chicago, IL 60612; Ponni Arunkumar, MD, Cook County MEO, 2121 W Harrison Street, Chicago, IL 60612; Lorenzo Gitto, MD, Sapienza University, Dept of Anatomy, Histology, Forensic Medicine, & Orthopedics, Viale Regina Elena, 336, Rome 00169, ITALY*

After attending this presentation, attendees will better understand the incidence, including seasonal trends, demographics, associated drugs, and significance of asthma history of heroin deaths.

This presentation will impact the forensic science community by providing data on heroin deaths in Cook County and a comparison of the demographics, autopsy, and histological findings of heroin users who had a history of asthma to those with did not.

According to the National Survey on Drug Use and Health (NSDUH), in 2012 approximately 669,000 Americans reported using heroin in the past year. The National Institute on Drug Abuse showed that in 2013 more than 8,000 deaths from heroin occurred in the United States.

Asthma is a chronic inflammatory disorder of the airways characterized by hyper reactivity, with reversible airflow obstruction, and respiratory symptoms of an attack that can include shortness of breath or respiratory distress even until death. Asthma is a commonly encountered disease in the United States, with an estimated 25.5 million people afflicted in 2012.

Although studies have shown a link between asthma deaths and heroin abuse, the process in which opiates exacerbate asthma is still unclear. Heroin may impair judgement during an acute asthma attack leading to inadequate treatment and late arrival for care. Alterations in mental status may increase aspiration risk and predispose to aspiration-induced bronchospasm. Some studies have demonstrated that opioid-induced bronchoconstriction is mediated by histamine release and that heroin itself can degranulate mast cells and release pre-formed mediators of inflammation.

The files of the Cook County Medical Examiner's Office in Chicago, IL, were searched for cases involving heroin as a primary or contributory cause of death from January 2013 to December 2014. Cases were reviewed for age, sex, race, cause and manner of death, gross and microscopic autopsy findings, and toxicology results. The route of administration of the drug was reported if determined during the death investigations.

Six hundred ninety-six cases were identified that met the criteria: 149 female and 547 male. The ages ranged from 17 years to 68 years of age. The race distribution was: 435 Caucasian, 257 African-American, 1 Oriental, and 3 Hispanic. The manner of death was determined to be accident in 681 cases, suicide in 5, natural in 5, homicide in 2, and undetermined in 3.

Of these cases, 662 listed heroin as the primary cause of death: 142 female and 520 male. In this group, the age range was the same as above. The smallest number of cases occurred in the month of January 2013 (17), while the greatest number occurred in September 2014 (37). Regarding seasonal distribution, it was found that in the spring (March-May of both years) the smallest number of deaths (154) occurred, whereas in the fall (September-November of both years) the greatest number (180) of deaths occurred.

In 34 cases, heroin was a contributory cause of death. In this subset, the age range was 20 years to 64 years old. Seven were female and twenty-seven were male. Regarding the seasonal distribution, in spring (March-May of both years), the greatest number of deaths (15) occurred, whereas in fall (September-November of both years) the smallest number (5) of deaths occurred. In this group, six cases died of "bronchial asthma."

In both of the groups, "heroin as primary cause of death" and "heroin as a contributory cause of death," this study found a history of asthma in 58 cases. In this subset, the age range was 19 years to 64 years old. Twenty-one were female and thirty-seven were male. Regarding the seasonal distribution, in the winter (January, February, December of both years), the greatest number of deaths (21) occurred, whereas in summer (June-July of both years) the smallest number (10) of deaths occurred.

Whenever lung slides were available, they were reviewed and graded for asthma changes.

This work supports the hypothesis that a history of asthma is frequently seen in heroin deaths. In these cases, deaths usually occur in the coolest months, perhaps because cold air acts as a trigger for exacerbations of asthma.

Even though there are a number of limitations (route of administration not always known, small number of cases, etc.), this study provides a review of heroin deaths in a large county in the United States.

Heroin, Asthma, Death

H102 Utility of Toxicology Screening in Older Adults Based on History and Scene Investigation

Matthew F. Fox, MD, Rush University Medical Center, 1653 W Congress Parkway, Chicago, IL 60612; and Steven M. White, MD, PhD, County Cook OME, 2121 W Harrison Street, Unit D7, Chicago, IL 60612*

After attending this presentation, attendees will gain a better appreciation for resource utilization with respect to toxicology screening in older (greater than 59 years of age) adults based on history and scene investigation.

This presentation will impact the forensic science community by highlighting the need for considering resource utilization with the judicious use of toxicology screening in older adults.

Toxicological testing is one of the most expensive, time-intensive, and labor-intensive aspects of death investigation. With increasing budgetary constraints, medical examiners and coroners are constantly evaluating budgets to decide where cuts can be made. Determining the cause and manner of death and completing the death certificate on the same day as the postmortem examination is ideal, if possible. In the past, the Cook County Medical Examiner's Office in Chicago, IL, performed an abbreviated toxicology panel consisting of screening tests for ethanol, opiates, and cocaine on every decedent examined. In people aged 60 years old or older, the toxicology screening tests are usually negative; however, occasionally, unexpected (no history of drug use and no drug paraphernalia present at the scene) positive drug screens occur after the cause and manner of death had been determined, necessitating amendment of the death certificate. This study was performed to determine how many positive drug screens occurred in people aged 60 years old or older occurred in the absence of a history of drug use (or drug paraphernalia present at the scene).

A search of the database of the Cook County Medical Examiner's Office for decedents aged 60 years old or older in 2012 (when toxicological testing was performed on every decedent examined), in which the cause of death was due to illicit drug use (including ethanol, opiates, and cocaine), was performed.

There were a total of 2,009 deaths in people over the age of 59 years old. Of these cases, 53 people died as a result of ethanol, opiate, or cocaine toxicity. The age range was 60 years to 89 years, with a mean of 65 years. The male:female ratio was 40:13. In 57% of the deaths, one or more drugs was listed as a cause, with the remaining 43% listed as a significant contributor. Including part I and II of the death certificate together, alcohol alone was listed in 17% of cases, cocaine in 38% of cases, and opiates alone in 15% of cases. Polypharmaceutical overdoses, which included combinations of ethanol, cocaine, and opiates, accounted for 23% of these cases. Only six cases (11% of the 53 cases) had no history of drug use and no paraphernalia was identified at the scene of death. Therefore, only 0.3% of decedents over the age of 59 years (n=2009) died from illicit drug use when there was no history of drug use or drug paraphernalia at the scene.

Toxicology, Screening, Elderly

H103 A Prospective Double-Blinded Comparison of Autopsy and Postmortem Computerized Tomography (PMCT) for the Evaluation of Potential Drug Poisoning Deaths

Ian Paul, MD, Office of the Medical Investigator, 1101 Camino de Salud, NE, Albuquerque, NM 87102; Sarah Lathrop, DVM, PhD, 4920 Edwards Drive, NE, Albuquerque, NM 87111; Gary M. Hatch, MD, University of New Mexico School of Medicine, Center for Forensic Imaging, MSC 07 4040, 1101 Camino de Salud, NE, Albuquerque, NM 87102; Chandra Y. Gerrard, BS, Office of the Medical Investigator, Rad-Path Ctr for Forensic Imaging, 1101 Camino De Salud, NE, Albuquerque, NM 87131; Valerie Poland, BA, Office of the Medical Investigator, 1101 Camino de Salud, NE, Albuquerque, NM 87102; Ross E. Zumwalt, MD, Office of Medical Investigator, 1 University of New Mexico, MSC 074040, Albuquerque, NM 87131-0001; Sam W. Andrews, MD, Travis County Medical Examiner's Office, 1213 Sabine Street, Austin, TX 78701; Jan Price, RN, MSA, AAAM Injury Scaling, PO Box 4176, Barrington, IL 60011; Gary W. Mlady, MD, Department of Radiology, University of New Mexico, Albuquerque, NM 87131; Jennifer W. Pohl, MD, PhD, Department of Radiology, University of New Mexico, Albuquerque, NM 87131; Brad W. Cushnyr, MD, Department of Radiology, University of New Mexico, Albuquerque, NM 87131; Philip W. Wiest, MD, Department of Radiology, University of New Mexico, Albuquerque, NM 87131; and Kurt B. Nolte, MD, Office of Medical Investigator, MSC07 4040, 1 University of NM, Albuquerque, NM 87131-0001*

After attending this presentation, attendees will understand how and when PMCT scans can supplement or supplant autopsy in potential drug poisoning deaths and how the two procedures compare in determining cause of death.

This presentation will impact the forensic science community by offering an alternative or supplement to autopsies for specific types of drug poisoning deaths.

In 2013, there were 589 drug poisoning deaths in New Mexico constituting 28% of the autopsies. In order to better understand when PMCT could be utilized to either replace or supplant traditional autopsies on potential drug poisoning deaths, autopsy reports and PMCT findings from a prospective cohort of these deaths investigated by the New Mexico Office of the Medical Investigator were collected between January 2013 and August 2013. Four hundred sixty cases were included. Autopsy reports and PMCT reports were completed, as was a study pathologist's Cause of Death (COD) statement (using PMCT, toxicology, and circumstantial information). Results were paired in Excel® spreadsheets for review in a consensus conference that included a radiologist and pathologist not involved in the original case assessment. The study was double-blinded.

The drug poisoning cohort included 307 males (67.2%) and 157 females (32.8%), ranging in age from 15 years to 90 years old. Accident was the most common manner of death (51.2%), followed by natural (32.4%), and suicide (10.1%). Males between the ages of 40 years and 69 years old were overrepresented.

More injuries and disease processes were recorded from the original autopsy than in the PMCT report used in the COD review. There were 7,121 findings, of which 2,734 were coded as matches (38.4%) and 5,349 (73.6%) as misses. Significantly more findings were ruled R1 (missed on PMCT and should have been seen) than were ruled A1 (missed on autopsy and should have been detected) (23.2% versus 15.3%, $p < 0.0001$). Similarly, significantly more PMCT findings were coded as R2 (would not expect to have seen on PMCT) than A2 (would not expect to be seen at autopsy) (30.7% versus 15.4%, $p < 0.0001$). The sensitivity of detecting all pathologic conditions and injures of PMCT in drug poisoning deaths was 65.5% and for autopsy was 74.3%.

Focusing on the COD statements, the first line of Part 1 of the death certificate was ruled a match and correct in both cases in 77.9% of drug poisoning deaths. In Part 2, Line 1 was correct and matched in the majority of deaths reviewed with a Part 2 (122/169, 72.2%).

Comparing COD evaluations in decedents under the age of 40 years old (113 decedents) to those for decedents ages 40 years and older (344 decedents), the first line of Part 1 of the death certificate was significantly more likely to match between the original autopsy and the CT-based reviewing pathologist ($p=0.019$) (85.8% vs. 75.3%). The first line of Part 2 was more similar between the two age cohorts, with no significant difference in percent matched and correct (63% and 74%, $p=0.24$).

Autopsy most frequently missed vascular calcifications, fractures, nephrolithiasis, aspiration, and diverticulosis, it was found. The findings most commonly ruled "A2" (not expected to be seen at autopsy) included vascular calcifications of the carotid bifurcations, degenerative changes, fractures, and osteoarthritis. The most commonly missed findings on PMCT included external contusions, cardiomegaly, obesity, and pulmonary edema. The most commonly reported R2 (not expected to be seen on PMCT) finding was "substance present on toxicology," followed by external abrasions, atherosclerotic stenosis, and hepatitis.

With very few acute injuries and a significant incidence of natural disease, drug poisoning deaths are challenging for approaching as external exam, toxicology and PMCT-only. Deaths that present initially as potential drug poisoning are often ultimately attributed to natural disease or an interplay between natural disease and drugs, especially in people over the age of 40 years old; however, in decedents under the age of 40 years old, the use of PMCT only resulted in 86% correct COD determinations. PMCT can certainly be used to supplement traditional autopsy findings in cases of drug poisoning and, with appropriate investigative information, PMCT can reasonably supplant autopsy in potential drug poisoning cases in people less than 40 years of age.

PMCT, Autopsy, Toxicology

H104 Heroin-Related Deaths in Denver, Colorado

Meredith A. Frank, MD, Denver OME, 660 Bannock Street, Denver, CO 80204*

After attending this presentation, attendees will understand the significance of the insidious nationwide rise in heroin abuse and heroin-related deaths. Attendees will also learn how this epidemic permeates the large urban community of the city and county of Denver, which has experienced a striking increase in heroin-related deaths over the last decade.

This presentation will impact the forensic science community by providing additional data for continued research of heroin-related deaths within the United States and its larger cities and by increasing the discussion among forensic professionals regarding heroin-related deaths. This increased discussion will hopefully lead to further epidemiological studies and assistance for programs or organizations to prevent such deaths.

Heroin use is reaching epidemic proportions in the United States as heroin use has greatly increased over the last decade among both men and women, most age groups, and all income levels, according to a recent report from the Centers for Disease Control. The increase in heroin use is understandably associated with a marked increase in heroin-related deaths. It is noted that between 2002 and 2013, the rate of heroin-related deaths nearly quadrupled, and in 2013 more than 8,200 people died due to heroin overdose in the United States.

Denver, CO, is the 21st largest city in the United States (population 663,862). At the Denver Office of the Medical Examiner (DOME), three board-certified forensic pathologists work to accurately determine cause and manner for unexplained, unexpected, and violent deaths in Denver. These forensic pathologists utilize investigation and autopsy, which is often supplemented by postmortem toxicologic studies and histology, in order to appropriately diagnose any contributing intoxication.

The DOME database includes a modified Systematized Nomenclature of Medicine (SNOMED) -based coding system for entry of major autopsy findings/diagnoses, as well as any contributing substances detected on postmortem toxicology within fields that are specific to the diagnoses or substances. The database can then be searched for cases with the presence of specific drugs and substances. To determine which deaths were potentially related to peri-mortem heroin use, a query of the database was performed. Codes for "heroin," "6-monoacetylmorphine" (6-MAM), and "morphine" were searched, and the related cases were reviewed; heroin-related deaths certified at DOME from January 1, 2000 to July 1, 2015, will be discussed.

The large majority of cases positive for heroin metabolites were considered due to intoxication, either solely due to heroin present in combination with other substances detected (i.e., certified as "mixed drug intoxication" or "combined drug toxicity"). Some deaths were determined to be due to conditions which were entirely unrelated to the detection of heroin metabolites; however, these cases were rare. Of note, the query function of the database allows certain demographic data to be collected as well; therefore, comparisons of age, gender, and race will be provided, and any significant trends among these categories within the heroin-related deaths in Denver will be addressed, if present. Any associated substances and their role in the deaths will also be discussed.

Heroin, Death, Urban

H105 Case Report: Fatal Use of a Suspected Herbal Medication

Tiffany O'Neill, DO, 1 Medical Center Boulevard, Winston-Salem, NC 27157; and Donald R. Jason, MD, JD, Wake Forest University, School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157-1072*

After attending this presentation, attendees will better understand the work-up of a suspected herbal medicine death.

This presentation will impact the forensic science community by increasing awareness of herbal medicine deaths in a time when herbal medication use is increasing.

Herbal medications fall into the category of traditional medicine. According to the World Health Organization, traditional medicine is "sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement, or treatment of physical and mental illness."[1] This may also include terms such as complementary and alternative medicine. Herbal medicines have been in existence for thousands of years, are widely used, and generally thought of as safe by the public. Approximately 30% of patients use some sort of herbal medication for various reasons, such as a holistic approach, improving their health, and relieving symptoms from other medications.[2]

Herbal medicines do not require approval from the Food and Drug Administration and are regarded as "dietary supplements" so they are "presumed safe," unlike pharmaceutical medications which undergo a more vigorous approval process. The manufacturer is required to list the ingredients of the herbal medication on the label and is furthermore in charge of making sure the product is safe for consumption.[3] According to the *New England Journal of Medicine*, approximately 70% of patients that ingest herbal medicines do not routinely tell their doctors they are taking an herbal medication, which could possibly result in deadly interactions between prescription medications and the herbal medication.[4] In the forensic pathology setting, this can result in problems of lack of a full medication knowledge which can hinder toxicology testing. Toxicology does not routinely test for herbal medications and additional testing may need to be requested. If the herbal medication is known, detection may still prove to be difficult because the levels of lethality are mainly unknown, with some levels being too low to be detected.

The case study of an 18-year-old Hmong woman who was two months postpartum from her fourth pregnancy will be presented. The Hmong culture uses many different plants as traditional medicines, some of which can be used as oral contraceptives. This is thought to have been the objective in this case. The internal examination was significant for diffuse pink bodily discoloration. Extensive toxicology testing was performed including organic acids, organic bases, organic neutrals, cyanide, and rhodamine-B. This presentation highlights the difficulties associated with a suspected herbal medicine death.

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Herbal Medicine, Rhodamine-B, Forensic Science

H106 Postpartum Non-Atherosclerotic Spontaneous Coronary Artery Dissection (NA-SCAD) Recurrence in Subsequent Pregnancies: A Case Report

Casey P. Bitting, DO, University of New Mexico School of Medicine, Univ of NM Health Science Ctr, Msc08 4640, Dept of Pathology, 1 University of NM, Albuquerque, NM 87104; and Ross E. Zumwalt, MD, Office of Medical Investigator, 1 University of New Mexico, MSC 074040, Albuquerque, NM 87131-0001*

After attending this presentation, attendees will be aware of the risk of NA-SCAD in subsequent pregnancies, the associated histological findings of SCAD, and the current etiological thinking behind this rare entity.

This presentation will impact the forensic science community by introducing the concept of recurrent SCAD in subsequent pregnancies, a previously unreported phenomenon.

NA-SCAD, or separation of the layers of the coronary arterial wall, is a rare cause of sudden cardiac death predominantly seen in young, healthy women. One retrospective single-center cohort study of 87 patients with angiographically-confirmed NA-SCAD identified a predilection for women (82%) with the most common female association being postpartum status (18%). The mean age of postpartum patients was 33 years and the mean postpartum time frame was 38 days. Of the 87-patient cohort, 91% presented with chest pain, 49% had ST-elevation myocardial infarction, and 14% had a life-threatening ventricular dysrhythmia. The left anterior descending coronary artery was the most common location for dissection (71%).

While intimal tears have been suggested as the inciting event of NA-SCAD, these tears are rarely found at autopsy. Another proposed model is supported by the location of the plane of dissection, which is commonly between the tunica media and tunica adventitia. In this model, disruption and bleeding of the vasa vasorum leads to intra-medial hemorrhage and hematoma formation. Axial propagation occurs due to ongoing hemorrhage and clot formation in the absence of an intimal tear. Luminal collapse ensues. Originally considered a reactive phenomenon, the presence of eosinophilic infiltrates in 50% of NA-SCAD, with their collagenase-containing granules, suggests their primary, or at least propagating, role in dissection.

Several mechanisms have been proposed for increased peripartum risk of SCAD. Progesterone excess has been associated with elastic fiber disarray and loss of acid mucopolysaccharide ground substance, while estrogen-associated release of matrix metalloproteinase has been shown to result in cystic medial necrosis. These combined hormonal effects have been proposed to result in a loss of structural support of the vasa vasorum with resultant susceptibility to rupture, especially in the setting of pregnancy-induced hemodynamic stress.

A 30-year-old Hispanic female with a past medical history significant only for two cesarean sections was driving with her two children, ages three months and three years, when her car was seen to slowly drift off the road and come to a stop. A bystander found the woman slumped in the driver's seat with no signs of trauma. Her two children were secured in the back seat. Emergency medical services responded quickly, found the woman to be in ventricular fibrillation, and performed cardiac defibrillation without success. The woman was transported to the closest regional medical center where additional unsuccessful attempts at cardiac resuscitation were performed. In addition to being three months postpartum, the decedent had complained of chest pain one week prior to death. She did not smoke or use prescription or illicit drugs.

External exam revealed a well-nourished, Hispanic female (BMI 22.9kg/m2). Autopsy revealed a heart of normal shape and size (230 grams) with 90%-99% luminal reduction of the Right main Coronary Artery (RCA) beginning just distal to the right marginal branch and continuing through the distal posterior descending branch. Close inspection of the RCA revealed a pinpoint lumen surrounded by clotted blood. Sectioning of the heart revealed an extensive, white fibrous infarction of the anterior left ventricle and interventricular septum extending from the apex to the level of the mitral valve cusps in the distribution of the Left Anterior Descending (LAD) coronary artery. The LAD itself had no grossly identifiable abnormalities and there was no coronary or other arterial atherosclerosis.

Histological examination of the heart confirmed extensive replacement of the left ventricular free wall and adjacent interventricular septum by a dense collagen scar. Examination of the RCA revealed near-complete luminal compression by an extra-luminal, antemortem thrombus associated with extensive adventitial destruction. Eosinophilic infiltration of the tunica adventitia with areas of adventitial necrosis, myxoid change, and adjacent perivascular fat necrosis was prominent. There was also scattered early formation of fibrovascular granulation tissue at the outer margins of adventitial dissection.

The remote myocardial infarction described in this patient is the result of NA-SCAD of the LAD in the peripartum period of the first pregnancy and it is proposed that this is the first case of recurrent SCAD in the setting of subsequent pregnancy. Therefore, monitoring survivors of pregnancy-associated NA-SCAD is strongly recommended. In the setting of autopsy, Magnetic Resonance Imaging (MRI) should be considered in the evaluation of young, healthy, and especially peripartum females who present with sudden, unexplained death.

Coronary, Dissection, Pregnancy

H107 Custodial Suicides: A Review of Suicides of Incarcerated Persons Investigated by the Harris County Institute of Forensic Sciences Over a Ten-Year Period

Sara N. Doyle, MD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; and Sharon M. Derrick, PhD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will better understand the demographic characteristics, circumstances of incarceration, scene findings, and autopsy findings in cases of people who commit suicide while incarcerated. Attendees will also become familiar with recommendations for postmortem examination of persons who die in custody.

This presentation will impact the forensic science community by providing a set of data that includes detailed characteristics of custodial suicides which may be useful in developing prevention recommendations for these types of in-custody deaths.

According to the Bureau of Justice Statistics, suicides account for approximately 30% of the deaths occurring in local jails and 5%-7% of deaths occurring in state prisons, with more than 6,000 people committing suicide while in local jails and state prisons in the United States from 2001-2012.[1] The rate of suicides in detention facilities is estimated to be approximately three times greater than that of the general population, with custodial suicides potentially having a significant negative financial impact on the detention facility in addition to the devastating emotional impact on the family of the inmate.[2] Various demographic, institutional, and clinical risk factors have been demonstrated to be associated with custodial suicides.[3] From 2005-2015, the Harris County Institute of Forensic Sciences investigated approximately 30 deaths of people incarcerated in jails, prisons, and holding facilities which were subsequently classified as suicides. As expected, hanging was the cause of death in the overwhelming majority of these decedents. Only one death, in which the decedent intentionally ingested prescription medications he had been stockpiling during his incarceration, was due to a cause other than hanging. The demographic characteristics of the decedents, scene and investigative findings including how the hangings were accomplished and types of ligatures used, and the circumstances of incarceration including type of facility and cell, duration of incarceration, and offense for which the decedent was arrested will be discussed.

Because many of these in-custody deaths understandably raise concerns of foul play and may be scrutinized by various investigative agencies, the postmortem examination must be exceptionally thorough, carefully documented, and performed by an independent agency to eliminate/reduce the appearance of bias. Special recommendations for postmortem examination of in-custody deaths and documentation thereof will be reviewed in detail.

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Custodial Deaths, Suicide, Hangings

H108 Deaths Associated With a November 2014 Snowstorm ("Winter Storm Knife") in Erie County, New York

Katherine F. Maloney, MD, 501 Kensington Avenue, Buffalo, NY 14214; Nicole A. Yarid, MD, Erie County Medical Examiner's Office, 501 Kensington Avenue, Buffalo, NY 14214; Janinne Blank, 501 Kensington Avenue, Buffalo, NY 14086; and Tara J. Mahar, MD, 4103 Coventry Green Circle, Williamsville, NY 14221*

After attending this presentation, attendees will better understand the causes of death during a recent snowstorm in Erie County, NY.

This presentation will impact the forensic science community by providing information concerning what types of death are to be expected when there is a large snowfall and how to prepare for such an occurrence. This information could be used to guide preventive measures in order to potentially save lives.

In November of 2014 over the course of three days, up to seven feet of snow fell across southern Erie County, NY, in a lake effect snowstorm named "Winter Storm Knife" by local officials. This was one of the largest snowstorms in the region in terms of not only the amount of snow but how fast it fell. There were travel bans throughout the region, and many people were trapped in their cars on roadways and could not be reached even by emergency personnel for hours if not days. Even after the snow stopped, it took several days to clear enough snow to reopen many of the roads, and there were millions of dollars of damage to local homes and buildings due to roof collapses. This storm was also one of the deadliest in the region in recent memory.

There were 12 deaths thought to be attributed to the storm that were reported to the Erie County Medical Examiner's Office. Five of these deaths were certified as accidental in nature, and the remaining seven were due to natural causes but associated with the snowstorm. These included four people who died of cardiac events while trying to clear snow from their homes or businesses, two people who needed routine urgent medical care but were unable to be reached by emergency medical services, two people who died of carbon monoxide poisoning while waiting in their cars for assistance, two people who died of exposure (one outside and one in an abandoned house), one person who was struck by a vehicle clearing snow, and one elderly person who died shortly after being emergently transported from one nursing home to another when the roof was deemed unstable.

As with most large snowfalls, cardiac death associated with shoveling was the most common cause of death; however, an unexpected cause of death in this particular storm was people who died in their cars from carbon monoxide poisoning. In both cases, unbeknown to the drivers, the tailpipes of the cars became occluded by snow while the cars were running, causing the passenger compartments to fill with the deadly gas. While there is general awareness in the region that exhaust pipes on houses need to be examined during large snowfalls to make sure they are clear, it is not common for snow to accumulate so rapidly that people would be trapped in their vehicles with enough snow on the ground to occlude the tailpipe. Increased awareness of this hazard during a large snowfall could save lives.

Snowstorm, Death, Autopsy

H109 Effects of Weather and Lunar Phases on Forensic Autopsy Case Load: A Four-Year Review

Matthew D. Cain, MD, University of Alabama at Birmingham, 619 S 19th Street, Birmingham, AL 35233-7331; and Daniel W. Dye, MD, Jefferson County Coroner/Medical Examiner Office, 1515 6th Avenue, S, Rm 220, Birmingham, AL 35233*

After attending this presentation, attendees will appreciate the effects weather and lunar phases have on forensic cases, including homicide, suicide, accident, and natural death rates.

This presentation will impact the forensic science community by proving or disproving the anecdotal evidence that weather and the full moon play a substantial role in forensic caseloads or types. This presentation shows the effects, or lack thereof, of temperature, weather events (such as rain or snow), seasonal changes, and moon phases on manner of death and caseload.

Changes in seasons, such as the "winter blues," have long been known to affect the mood of individuals. In fact, medical diagnoses, such as Seasonal Affective Disorder, exist because of the significant impact weather has on some individuals.[1] Furthermore, weather events that decrease the amount of sunlight, such as rain, can exacerbate symptoms.[1]

In this study, information was extracted from the Jefferson County Coroner/Medical Examiner Office database regarding the number of cases and manner of death from 2010 to 2014. This data was merged with weather data collected for Birmingham, AL, as well as moon phase data.[2,3] In total, there was evaluation of 3,756 cases. For the preliminary temperature statistics, average temperatures were grouped into 10° increments (i.e., 31°F-40°F). This categorical data was paired with manner of death data and evaluated with an Analysis of Variance (ANOVA) in Statistical Analysis System (SAS). Seasonal data was analyzed using a Chi Goodness of Fit. Moon phases and weather events were evaluated by t-tests.

No statistical significance was observed when categorized temperatures were compared to homicide, suicide, accident, and natural deaths ($p=0.97$, 0.33, 0.87, and 0.64, respectively). Accidents (0.85 cases/day) followed by natural death (0.62 cases/day) were the most common manners of death. The evaluation of weather events, such as rain or snow, demonstrated an increased suicide average (0.24 versus 0.20 cases/day); however, this was not statistically significant ($p=0.097$). Seasonal data revealed a weak trend of increased spring accidents (0.92 versus total average 0.85 cases/day), but was not statistically significant ($p=0.08$). Lunar affects were initially examined by checking for statistical differences between full moon, new moon, and other phases. This examination showed a slight increase in suicide rate for the day of a full moon (0.27 versus 0.21 cases/day), but this was not significant ($p=0.34$). This was followed by examination of an approximate full moon range, two days before and after the full moon, and this showed almost no deviation from any other moon phase.[4] Finally, Sunday had the heaviest case load and was significant (2.3 total cases/day, $p=0.005$). This increase was due primarily to increased homicide and accident rates.

These findings indicate that weather patterns, moon phases, and seasonal changes do not significantly impact the trends for homicide, suicide, accidents, or natural deaths. Weather events, such as rain and snow, showed a slight increase in the suicide rate, but not to a significant level. The dataset does indicate that homicide and accident rates are increased on Sundays. While anecdotal accounts about the "full moon" or "summer heat" will continue to abound, this data shows these ideas are unfounded myths.

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Forensic Autopsy, Moon Phase, Weather Effect

H110 Trends in Officer-Involved Firearm Deaths in Oklahoma From 2005 to 2014

Kyla M. Jorgenson, MSc, OCME - Oklahoma, 1115 W 17th Street, Tulsa, OK L7L 6M8; Andrea L. Wiens, DO, OCME, 1115 W 17th Street, Tulsa, OK 74107; Eric Pfeifer, MD, 901 N Stonewall, Oklahoma City, OK 73117; and Joshua Lanter, MD, 3167 E 144th Street, S, Tulsa, OK 74008*

After attending this presentation, attendees will be able to identify trends associated with officer-involved shooting deaths in the state of Oklahoma from 2005 to 2014. Attendees will be able to evaluate trends over this ten-year period in the number of such fatalities each year and the average number of gunshot wounds identified at autopsy.

This presentation will impact the forensic science community by establishing true numbers of strictly firearm-related fatalities involving law enforcement officers. This will provide the public and forensic community with unbiased statistics about the decedents, death circumstances, and postmortem findings that can then be used to enhance public and officer safety.

Introduction: The purpose of this study was to examine the trends associated with officer-involved shooting deaths in the state of Oklahoma over a ten-year period and to establish the number of these deaths that occur each year. These deaths were analyzed to determine if officer-involved firearm deaths are on the rise and if the number of gunshot wounds is similarly increasing. This is deemed necessary due to the prominence of officer-involved firearm fatalities discussed in the media and its perception that Oklahoma has high numbers of such fatalities.

Methods: A query of homicide deaths in the state medical examiner's database was conducted. Through retrospective review, the case files of deaths due to shootings by officers of the law were examined for the decedent's demographics, circumstances of death, and postmortem findings.

Results: From 2005 to 2014, 142 deaths were identified as being caused by one or more gunshot wounds from a law enforcement firearm. Decedents were primarily male (95%, n=135), White (62%, n=62), and between the ages of 30 years to 39 years (35%, n=50) or 20 years to 29 years (30%, n=42). Deaths most commonly occurred in a residence (30%, n=43) or a roadway (25%, n=36) and involved one (21%, n=30) or two (24%, n=34) gunshot wounds. The number of officer-involved firearm fatalities was found to significantly increase (p=0.018) over the ten-year period, particularly due to a significant increase between 2009 and 2014 (p=0.002); however, while there appears to be an increasing trend in the average number of gunshot wounds identified at autopsy, this is not a significant increase (p=0.495).

Conclusions: As hypothesized, the number of individuals killed in officer-involved shootings has increased, with the number of deaths almost quadrupling from 2009 to 2014. Conversely, there has not been an associated increase in the number of gunshot wounds inflicted by law enforcement in these deaths. With increased prevalence of officer-involved firearm fatalities, there is an increased need for a formal tracking system to facilitate public and officer safety.

Forensic Pathology, Homicide, Officer-Involved Shooting

H111 Emergency Management, Death Investigation, and Pathology of a Mass Fatality Industrial Workplace Accident: The La Porte, Texas, Dupont® Plant Incident

Pramod Gumpeni, MD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; Jason M. Wiersema, PhD, Harris County Institute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054; and Allison Woody, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will better understand the emergency management response and death investigation response required to properly investigate and contain large-scale industrial accidents. This presentation will cover scene investigation, response, and pathologic findings in a methyl mercaptan leak.

This presentation will impact the forensic science community by increasing awareness of collaborative efforts between law enforcement, industrial agencies, and medical examiner offices in responding to multiple fatalities at chemical and industrial sites. This presentation will also shed light on a rare form of chemical asphyxia by methyl mercaptan.

The Harris County Institute of Forensic Sciences (HCIFS) provides forensic science services to all of Harris County and several adjoining county jurisdictions. Part of the county contains a large industrial complex that is part of the Houston Ship Channel. The Ship Channel and its related industries are home to several chemical industrial companies and their production plants. HCIFS sees many industrial workplace deaths every year, ranging from blunt trauma to asphyxia deaths.

In November 2014, a chemical leak occurred at a DuPont® production facility that was producing chemical pesticides. During the course of the leak and the subsequent evacuation, it was noted that several employees were not accounted for and were presumed dead inside the chemical facility.

In conjunction with Houston Fire Department, La Porte Police Department, and Channel Industries Mutual Aid, HCIFS mobilized an emergency response team in order to respond to the scene and assess the situation. By working with law enforcement and other government agencies, the Hazardous Materials (HAZMAT) situation was assessed and the decedents retrieved in an efficient and safe manner, without compromising the quality of the investigation. Coordination, communication, and collaboration with outside agencies was of paramount importance in order to ensure the safety of the investigative agencies and first responders.

The chemical that was of major concern was methyl mercaptan, which is an odorless agent used in propane preparation as well as in pesticide production. There were four deaths associated with this incident, all of whom were employees with the chemical plant. All decedents underwent full autopsies at HCIFS, and volatile toxicology testing was performed on all of the decedents. The cause of death in all of the decedents was "Asphyxia due to Exclusion of Oxygen," the manner "Accidental."

HCIFS worked with the National Chemical Safety Board in order to ascertain prior deaths due to methyl mercaptan and to assist with their investigation of the industrial plant. Information on a prior industrial accident involving methyl mercaptan was reviewed in order to ascertain proper toxicology testing and to help guide the forensic pathologist in determining pathologic findings.

By coordinating with first responders and HAZMAT teams for the investigation and decedent recovery, and with regulatory agencies during the examination and subsequent follow-up, HCIFS was able to successfully handle an industrial accident involving four decedents in a possibly dangerous recovery situation.

Forensic Emergency Management, Industrial Accidents, Death Investigation

H112 Cadaver Gravesoil Microbial Profiles During Decomposition

Sheree J. Finley, MS, Alabama State University, 915 S Jackson Street, Montgomery, AL 36104; Jennifer L. Pechal, PhD, Michigan State University, 243 Natural Science Bldg, East Lansing, MI 48824; M. Eric Benbow, PhD, Michigan State University, Depts of Entomology & Osteopathic Med Specialties, 288 Farm Lane, East Lansing, MI 48824; Boakai K. Robertson, PhD, Alabama State University, 915 S Jackson Street, Montgomery, AL 36104; and Gulnaz T. Javan, PhD, Alabama State University, Forensic Science Program, 915 S Jackson Street, Montgomery, AL 36104*

After attending this presentation, attendees will understand how to use high-throughput next generation sequencing analysis to characterize microbial taxa present in gravesoil associated with human bodies decomposing in a natural setting. Specifically, attendees will learn methodologies to use microbial (Bacteria and Archaea) taxa relative abundances to determine predominant community shifts throughout decomposition time.

This presentation will impact the forensic science community by informing practitioners interested in using high-throughput next generation sequencing of the microbial communities found in gravesoils how to develop a framework for determining decomposition time.

The goals of this study were to identify microbial communities associated with cadaver gravesoil and to classify the fluxes in predominant microbes throughout decomposition time. Soil microbial communities associated with decomposition in a natural habitat were characterized using the 16S ribosomal RNA (rRNA) gene from multiple soil samples associated with 18 sets of decomposing human remains at various stages of decomposition. The gravesoil samples were obtained from bodies placed above ground or buried in shallow graves (approximately 45cm) in a natural setting at the Forensic Anthropology Research Facility (FARF) at the Forensic Anthropology Center at Texas State (FACTS) University in San Marcos, TX. The corpse placement/burial times ranged from a three-day placement on the soil to 303 days burial in the soil. Several within-community species diversity indices (i.e., Shannon-Weiner, Simpson, and Whitaker's beta) were evaluated based on the microbial community structure of the cadaver-soil microorganisms as a function of time on or in the soil.

The results of this study demonstrated that the microbial communities differed according to the time that a corpse was placed on or buried in the soil. For surface-placed cadavers, Shannon-Wiener diversity decreased approximately 32% over time, while Whitaker's beta diversity showed that microbes in the soil increased during the process of decomposition; however, the beta diversity of soil microbial communities associated with buried cadavers was U-shaped, suggesting a non-linear relationship with deep soil communities and decomposition. Soil samples collected above buried bodies had more similar community compositions irrespective to the decomposition time. The results also documented that Proteobacteria, Actinobacteria, and Acidobacteria were the most predominant phyla detected in all stages of decomposition in all samples — surface placed, buried, and control. There was also a substantial reduction in acidotrophic bacteria (Acidobacteria) and edaphic bacteria (Verrucomicrobia) over decomposition. These novel outcomes describe microbial compositions across all stages of decomposition and support recent studies advocating that the discovery of succession patterns in microbial communities may be crucial to the advancement of the knowledge of human decomposition with potential applications in the forensic sciences.

This study provides novel microbial metagenomic information that may have the potential to be used to estimate time of death in a natural environment. The study demonstrates a technique that will soon meet the demand for rapid and reproducible methods in investigative forensic science using state-of-the-art genomics techniques to monitor the microbial community signatures in gravesoil.

Microbial Diversity, Cadaver Soil, Next Generation Sequencing

H113 The Influence of Predator Presence and Habitat Type on Blow Fly Oviposition

Kristi Bugajski, PhD, 1610 Campus Drive, E, Valparaiso, IN 46385*

After attending this presentation, attendees will be informed concerning oviposition timing differences between forest and prairie habitats. In addition, data will be presented on the impact of predators (Hymenoptera: Vespidae) on blow fly oviposition.

This presentation will impact the forensic science community by presenting a study in an area in which very little previous work has been performed. Blow flies are usually the first insects to arrive at a crime scene, so information about their oviposition is crucial for accurate postmortem interval estimations.

Forensic entomology uses data derived from insects to aid in criminal investigations. Blow flies (Diptera: Calliphoridae) are usually the first insects to arrive at a crime scene, often within minutes after exposure.[1] Their quick appearance on carrion is the foundation for Postmortem Interval (PMI) estimations.[2] Two areas that could potentially affect oviposition timing, and therefore PMI estimations, are the habitat carrion is in, and if there are blow fly predators present. This study examined the effect of habitat (prairie vs. forest) and the presence of predators (Hymenoptera: Vespidae) on blow fly oviposition timing.

Research was conducted from June 1, 2015, through August 10, 2015, at Pierce Cedar Creek Institute in Barry County, MI. Forest and prairie habitats were compared in observation trials. Ten bait cups were used in each of nine paired trials. Each cup consisted of a 453g clear plastic cup with 6mm of vermiculite in the bottom and a foil cup with approximately 60g of aged chicken liver. The cups were covered before they were placed in the field in one of three paired prairie and forest sites, with five bait cups per habitat. The covered cups were placed on the ground at the sites four hours after sunrise. Six hours after sunrise, the lids were removed from the cups and observations began. Every half hour the cups were checked for blow fly eggs and the presence of blow flies and other insects. Once blow fly oviposition was observed, the cup was covered, labeled, and removed from the field. Observations ended 12 hours after sunrise. Bait cups with blow fly eggs or flesh fly larva were placed in a fume hood and reared to the third larval instar stage and identified.

The manipulation studies were all conducted in the same forest location. Nine bait cups, as described above, were used for each of ten manipulation trials. Three bait cups contained chicken liver and served as controls. Three bait cups had a pinned wasp placed on the chicken liver to serve as a visual manipulation. An odor manipulation consisted of crushing two wasps and sprinkling the crushed pieces over the chicken liver. Bait cups were covered and randomly placed using a random number generator into yellow platform stands in the fields at four hours after sunrise. Observations started at six hours after sunrise and were conducted as described above.

Data will be analyzed using Statistical Package for the Social Sciences (SPSS) statistical software. Preliminary data suggests there is no significant difference in habitat preference between prairies and forests. Oviposition occurs on average of one hour earlier in the prairie than the forest locations. Preliminary results suggest that the presence of pinned wasps and crushed wasps does not impact oviposition occurring, but could impact the timing. Live wasps that were observed on bait cups did impact oviposition. Blow fly behavior observations and final results will be included in the presentation.

Any information on blow fly oviposition timing is critical for accurate PMI estimations. A one-hour difference in oviposition doesn't sound significant, but could have an impact on criminal investigations.

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Blow Fly, Forensic Entomology, Predator Presence

H114 Dynamics of Necrophagous Insect Species and Bacteria From Swine Carcasses During the Warm Season in Romania

Lavinia Iancu, PhD, Grigore Antipa National Museum of Natural History, Sos. Kiseleff, Nr 1, Bucharest 011341, ROMANIA; and Cristina Purcarea, PhD, Institute of Biology Bucharest, 296 Splaiul Independentei, Bucharest 060031, ROMANIA*

After attending this presentation, attendees will better understand corroborated entomological and microbiological approaches by gaining insights on the dynamics of necrophagous insect species and potential microbial decomposition biomarkers identified from swine carcasses during the summer months.

This presentation will impact the forensic science community by demonstrating the importance of using both entomological and microbiological methods, with the goal of narrowing the biases in postmortem interval estimation in death investigations.

The concept of forensic entomology dates back to the 13th century, being mentioned for the first time in China by Sung Tzu. Since then, this method has developed worldwide, demonstrating the importance of using necrophagous insect species as physical evidence for postmortem interval estimation. In Europe, forensic entomology has been recognized by forensic experts since the 19th century, expanding into countries such as the United Kingdom, France, Germany, Italy, Poland, the Netherlands, Austria, Spain, and Switzerland. At present, the entomology method is not included among the forensic expertise in Romania. Moreover, the identification of bacterial communities from decomposed carcasses represents an early stage in worldwide scientific investigations, with few studies attempting to identify the microorganisms in order for them to be used for the postmortem interval estimation.

Therefore, this study represents the first experimental research monitoring the carcass decomposition process during the warm season in the urban area of Bucharest, Romania, by using corroborated entomological and microbiological approaches to identify the diversity and dynamics of insect and bacterial taxa sampled from swine carcasses during the warm season.

The experiment lasted 14 weeks (July 10, 2013-October 10, 2013), covering the summer and beginning of the autumn months. Three swine carcasses were mounted outdoors, directly on the ground at a distance of 20m from each other and protected by metallic cages. The environmental parameters were constantly recorded throughout the entire survey.

Necrophagous insect species, both adult and immature stages, were sampled daily from the swine carcasses and identified by taxonomic and genetic methods. In order to identify the bacterial taxa associated with the swine carcasses, tissues were sampled from the colon (rectum) and mouth cavities using a sterile metal loop. The tissue sampling protocol comprised ten occasions throughout the decomposition. The identification of bacterial communities was carried out by Denaturing Gradient Gel Electrophoresis (DGGE) analysis of the 16S ribosomal RNA (rRNA) gene fragments.

The necrophagous insect species succession comprised in the first colonization wave was the Calliphoridae and Muscidae species, followed by Sepsidae, Staphylinidae, and Dermestidae. The dynamics of both dipterans and coleopterans was in part biased given the dominant presence of *Crysomya albiceps* (Diptera: Calliphoridae) which massively colonized all three carcasses, with up to 2,000 larvae being sampled. The presence of this species had a negative impact on the time presence of other sympatric insects and starting with weeks three and four, no other dipteran species apart from *C. albiceps* was observed on the carcasses. At the same time, Coleoptera was poorly represented by only two species, *Dermestes undulatus* (Dermestidae) during the skeletal-remains stage and predacious *Creophilus maxillosus* (Staphylinidae). The relative abundance of the insect species adults and immature stages was assessed and their presence was recorded during the entire decomposition process, with the environmental condition variations also being considered.

The diversity of bacterial taxa from the swine tissues revealed by the DGGE profile indicated the presence of 26 taxa from the colon (rectum) and 22 taxa from the mouth cavity, respectively. The dynamics of bacterial communities from these cavities indicated two time tendencies, distinguished in the first and last weeks of experimentation. Firmicutes representatives were dominant in both cavities, closely followed by Gammaproteobacteria. The bacterial succession during the decomposition process could be in correlation with the environmental conditions, swine decomposition stages, and insect presence. Moreover, bacterial taxa were identified from ten *C. albiceps* larvae. Two insect-specific bacterial species were also identified from both the mouth and colon (rectum) cavities of all three swine, leading to a possible identification of potential microbial biomarkers that can be used for postmortem interval estimation.

This pioneering study on Romanian territory represents the first experimental investigation of entomological and microbiological diversity and dynamics from decomposed swine carcasses and attempts to demonstrate the importance of introducing forensic entomology as a valid method in this country, adding data to current knowledge regarding the bacterial taxa involved in decomposition.

Forensic Entomology, Microbiology, Swine Carcasses

H115 Postmortem Community Dynamics of the Larval Mass Microbiome

Emily Junkins, BS, Chaminade University of Honolulu Forensic Sciences, 3140 Waialae Avenue, Honolulu, HI 96816; and David O. Carter, PhD, Chaminade University of Honolulu, Forensic Sciences Unit, Honolulu, HI 96816*

After attending this presentation, attendees will understand the microbial community dynamics involved in carcass decomposition and its potential use as a forensic tool.

This presentation will impact the forensic science community by exploring an unstudied microbial habitat and identifying trends that will aid in estimating Postmortem Interval (PMI).

Recent studies have begun to characterize microbial community dynamics of the skin, soil, gut, and oral cavities during carcass decomposition. One area that has yet to be explored is the larval mass that forms after flies oviposit on carrion. These masses have predictable traits that are of forensic value; for example, the development of these larvae can be used to estimate PMI. The goal is to determine if some of these predictable traits are microbial. The microbiology of this niche will provide novel insight into a common decomposition tool and a regularly encountered phenomenon in death investigation. The current project hypothesizes that the maggot mass, like the carcass as a whole, has a specific decomposer community that changes over time. This project proposes that these changes are linked to both taxonomic abundances and the chemical environment.

Three swine carcasses (*Sus scrofa domesticus*), killed via electrocution, were decomposed in a tropical savanna ecosystem located in Palalo Valley, Oahu, HI, in June 2014. Relative humidity (%) and temperature (°C) were recorded at intervals of one hour. Sampling commenced at 74h postmortem when larval masses were established and at 80h, 98h, 104h, 122h, and 128h postmortem; five swabs were non-destructively sampled at each sampling time. Swabs were immediately placed in sterile tubes and frozen at -20°C. Once the survey was complete, the swabs were subjected to 16S metagenomic sequencing. In addition, chemical readings of the maggot mass were obtained *in situ* using a portable meter with sensors to measure temperature, pH, and oxidation-reduction potential (Eh).

The structure of the larval microbial community shifted whereby three distinct microbial communities were detected, hereinafter referred to as pre-98h, 98h, and post-98h postmortem. The pre-98h community was dominated by phyla Firmicutes (~89%) and Proteobacteria (~19%), both of which were significantly ($p < 0.0001$) more abundant than all other phyla. At 98h postmortem, the abundance of phylum Proteobacteria increased to represent ~77% of the community, while phylum Firmicutes decreased to ~23%. Both phyla remained significantly ($p < 0.0001$) more abundant than other phyla. As PMI progressed beyond 98h, Firmicutes steadily increased and Proteobacteria steadily decreased. Firmicutes dominated (~97%) this final community; it was significantly ($p < 0.0001$) more abundant than all other phyla. This shift was also observed at each taxonomic rank. Further, it was found that Firmicutes and Proteobacteria were significantly ($p < 0.0001$) negatively correlated over time ($R^2=0.999$; $y=-1.02x + 100$).

These shifts can be explained by biotic and abiotic factors involved in the larval life cycle. The pre-98h maggot mass was one of the least alkaline (7.32pH) but most reducing (-295.5mV) environments of this study. This is most likely due to the breakdown of macromolecules into their amino acid constituents and the activity of many lactic acid (*Enterococcus* sp., *Lactobacillus mucosae*) and sulfur/sulfate reducing (*Pseudomonas* spp., *Desulfovibrio* spp.) bacteria lessening the pH and Eh. As larvae grow, more oxygen may diffuse into the maggot mass, decreasing the Eh (-285mV) and increasing the pH (7.45). The drastic shift observed at 98h postmortem could have been achieved in a few different ways. One, this environment had the capability to support aerobic life, which could mean that *Clostridium* spp. (Phylum:Firmicutes) were unable to grow and/or members of family Xanthomonadaceae (Phylum:Proteobacteria) were able to outcompete. Secondly, as the larval mass continued to consume the carcass, adult flies were inoculating the larval mass with Xanthomonadaceae. The final phase was dominated by phylum Firmicutes (~97%) and was more similar to a microbial community indicative of the surrounding environment. For example, *Clostridium* spp. are saprotrophic organisms that are ubiquitous in soils. Overall, a significant ($p < 0.0001$, $F=397$) interaction between time and taxa was observed and it appeared to affect only Firmicutes and Proteobacteria.

Taphonomy, Decomposition, Entomology

H116 Indoor vs. Outdoor Forensic Entomology: Exploring the Differences, Challenges, and Opportunities of Indoor Scenes

Michelle R. Sanford, PhD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will better understand the differences, challenges, and opportunities presented by indoor forensic entomology scenes and cases.

This presentation will impact the forensic science community by highlighting this understudied area of forensic entomology casework, which will allow for more thorough observations at scenes and more intensive evaluation of forensic entomology reports of indoor scenes. This presentation will also highlight areas for future research in the area of forensic entomology.

Forensic entomology in the medical examiner's office involves the collection, identification, and evaluation of insects and related organisms from decedents whose deaths are being investigated by the medical examiner. These cases often involve the collection of insects that have colonized decomposing decedents during the course of the medicolegal death investigation and need not be limited to homicide investigations but can include any manner of death. Estimation of the Time Of insect Colonization (TOC) of human remains can be used to estimate the Postmortem Interval (PMI) for the decedent.[1]

In this presentation, forensic entomology casework data from indoor scenes analyzed by the Harris County Institute of Forensic Sciences (HCIFS) will be presented, highlighting several areas of interest to the interpretation of forensic entomology specimens and data. The forensic entomology cases from January 2013 through June 2015 consisted of 67% indoor scenes, 31% outdoor scenes, and 2% from hospitals (myiasis cases), illustrating the large percentage of indoor scenes. The presence of the body indoors significantly influences the insect community, the temperatures experienced by the insects, and the potential interpretation of TOC and how accurately it reflects the PMI. Humans typically alter the temperature of the indoor environment through the use of heating, air conditioning, or by other means, illustrating the potential difficulty in using weather station data to approximate indoor temperatures when calculating the development of the colonizing insects. The presence of the body indoors has been suggested as a cause of delayed colonization; however, the impact of this delay in practice is unknown, particularly when factors such as hoarding or pets are introduced to the scene.[2-4] Case data from indoor scenes also present the opportunity to use differences in insect community composition to potentially identify movement of decedents.[3-5] In Harris County, TX, flesh flies (Sarcophagidae), including the commonly encountered species *Blaesoxipha plinthopyga*, were encountered in 52.5% of forensic entomology cases (N=139 through June 2015). Sarcophagidae were encountered in 94.5% of cases from indoor scenes and only 5.5% were from outdoors. Phoridae, including the scuttle fly, *Megaselia scalaris*, were found in 37.4% of the forensic entomology cases for this same period and were encountered in 96.2% of the cases from indoor scenes and only 3.8% of the cases from outdoors. Blow flies (Calliphoridae) were encountered in 64.0% of the forensic entomology cases, 55.1% of the cases from indoor scenes, and 44.9% of the cases from outdoor scenes. This suggests that under certain circumstances, finding Sarcophagidae and/or Phoridae on a body indoors or outdoors might indicate possible body movement and the possibility of an additional scene.

The abundance and importance of indoor scenes has become evident in the forensic entomology casework analyzed by the HCIFS. Indoor scenes present new challenges and opportunities not only for methods development and research but also for more intensive observation of insects at indoor scenes and more critical interpretation of forensic entomology reports based on indoor scenes.

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Sarcophagidae, Phoridae, Decomposition

H117 The Utility of Soil Eukaryotes During Human Decomposition and Their Potential Forensic Applications

Vanessa Sufrin, MS, 11507 Harvestdale Drive, Fredericksburg, VA 22407; Tawni L. Crippen, PhD, Agricultural Research Service, U.S. Dept of Agriculture, College Station, TX 77845; Jeffery K. Tomberlin, PhD, TAMU 2475, Dept of Entomology, College Station, TX 77843-2475; Aaron M. Tarone, PhD, Department of Entomology, College Station, TX 77843; Jennifer L. Pechal, PhD, Michigan State University, 243 Natural Science Bldg, East Lansing, MI 48824; M. Eric Benbow, PhD, Michigan State University, Depts of Entomology & Osteopathic Med Specialties, 288 Farm Lane, East Lansing, MI 48824; and Baneshwar Singh, PhD*, Virginia Commonwealth University, Dept of Forensic Science, 1015 Floyd Avenue, Rm 2015, Richmond, VA 23284*

After attending this presentation, attendees will better understand how the eukaryotic community changes in soil during the human decomposition process and how this information can be used for forensic purposes. In addition, attendees will also be updated on 18S rDNA Ion Torrent™ Sequencing and associated data analysis approaches developed for the determination of eukaryotic community structure in soil associated with human cadavers.

This presentation will impact the forensic science community by providing detailed information on key eukaryotic groups in soil under decomposing human cadavers, whose changes in relative abundance may potentially be modeled for the prediction of time since death.

For more than a century, soil use in criminal investigations has been limited to its chemical and physical properties; however, with the advent of deep sequencing technologies, the microbial communities in the soil associated with human cadavers have the potential to be used in several forensic applications.[1] Many studies have addressed the changing chemical composition of soil beneath a decomposing cadaver; while a few have highlighted the effect this has on the life in the soil, none have yet described the change in the eukaryotic community.[1-3] This study investigated how soil eukaryotic communities change when exposed to human decomposition and the potential use of that change in forensic applications.

To achieve this, DNA was extracted using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) DNA extraction method from soil collected beneath and 1m away from six human (three with insect access and three with no insect access) and three porcine remains, every day for five days; the samples collected 1m away from the remains served as the control.[4] DNA was also extracted from eight of the cadaver sites at a later time, resulting in samples collected between 50 and 415 days since placement. Extracted DNA was amplified and sequenced for variable regions 8 and 9 of the 18S rDNA using Ion Torrent™ semiconductor sequencing following the manufacturer's protocol. Sequences were analyzed using the Mothur pipeline for hierarchical classification.[5] Resulting data was converted to percent abundance, a square root transformation was applied, then both Analysis of Similarities (ANOSIM) and Analysis of Variance (ANOVA) statistical analyses were performed, where appropriate.

Within 5 days after placement, four out of the nine cadavers had significantly different eukaryotic community structure in the soil beneath the remains when compared to the controls. Differences in percent abundances of eukaryotic taxa were also observed between samples collected beneath the remains within 5 days to those that were collected 50 days to 415 days after placement of the remains. The differences seen between soil samples collected beneath and 1m away from cadavers over the first 5 days cannot be attributed to the decomposition process — too much variation existed within the control soils to comfortably suggest that the changes were a direct result of the decomposition process; however, the fact that eukaryotic community structure looks very different for soil samples collected beneath decomposing bodies 50 days to 415 days after being laid out does show that changes are occurring.

In conclusion, this study provides evidence that the eukaryotic community associated with soil beneath human cadavers can help in the identification of decomposition sites and in estimation of the postmortem interval.

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Eukaryotes, Forensics, Soil

H118 Heat Signatures Produced by Maggot Masses: Using Forward Looking Infrared Radar (FLIR) Mounted on a Helicopter to Locate Human Remains

Ian Dadour, PhD, Boston University, Program in Forensic Anthropology, Dept of Anatomy & Neurobiology, Boston, MA 02118; and Michael Lee, PhD, Western Australian Police, Forensic Division, 2 Clayton Road, Midland 6056, AUSTRALIA*

After attending this presentation, attendees will better understand how maggot masses as part of the decomposition process can be detected using FLIR mounted on a helicopter.

This presentation will impact the forensic science community by providing results from a series of controlled experiments in an area of limited research. This presentation will demonstrate how FLIR is a useful tool for the detection of decomposed remains in scenarios ranging from deceased missing persons to homicides.

FLIR is an established search and rescue method for locating people by using their heat signature. Maggot masses are known to generate significant heat and this study set out to establish whether FLIR could be utilized to locate surficial human remains via the heat signature generated by the maggot masses. If the FLIR could detect the maggot masses, over what time period were they visible?

Hand-held thermal imaging cameras at close range have been used to record larval aggregation temperatures. In 2014, a study using hand-held thermal imaging cameras were used to detect the heat signature of larval aggregations of pig cadavers. This research found that the larval aggregations were visible to the thermal imaging camera over a certain period of time and over a relatively short distance (maximum distance described as ~35m). Even more recently in 2015, a helicopter-mounted FLIR was used to locate two cadavers in a field in Duisburg, Germany, during the summer months. The results of this study showed that the larval aggregations were visible via FLIR for a period of up to 20 days.

The current experiment was carried out in a research facility south of Perth, Western Australia, under early autumn and winter conditions. Pig cadavers were utilized as human analogues. During the autumn, the weather was particularly hot and dry with daytime temperatures ranging from 30°C to 40°C. The nighttime temperatures remained fairly high, ranging from 9°C to 19°C and throughout the experiment it did not rain at any time. The site was visited to conduct measurements on 23 separate occasions, mostly between 7:00 p.m. to 10:00 p.m. and the helicopter visited on six different occasions. During the winter period, the trial site experienced maximum daytime temperatures ranging between 15°C and 24°C (with an average temperature of ~19°C). Nighttime temperatures were much cooler, ranging between 2°C and 15°C (with an average temperature of just ~5°C). On several occasions, the area was subject to significant rain and storms.

During the winter experiment, the site was visited to conduct measurements on 42 separate occasions, between 7:00 p.m. and 11:00 p.m. The helicopter visited the trial site on 22 occasions during the winter trial, 5 times during daylight hours and the remaining times during the evening.

Ground measurements included temperature data, insect activity, and the ambient soil temperature recorded from the cadavers.

The results of this study showed that the heat generated by maggot masses in surficial remains was visible to the FLIR. During the autumn, the results show that between day 3 and day 6, the heat signature generated by the larval aggregations were highly visible. By day 7, the cadavers were slightly less visible to the FLIR operator, but nonetheless still identifiable. Between day 14 and day 20, the cadavers remained only weakly visible. In contrast, during the winter trial, the larval aggregations did not develop until between day 9 and day 13; however, once the larval aggregations established themselves, they were present for a period of 14 days on average.

FLIR, Maggot Masses, Decomposition

H119 Statistical Confidence Limits for a Prediction of Carrion Insect Age Based on a Categorical Response Variable

Lynn R. LaMotte, PhD, Louisiana State University, Health Sciences Center, School of Public Health, 2020 Gravier Street, New Orleans, LA 70112; Amanda L. Roe, PhD, College of Saint Mary, 7000 Mercy Road, Omaha, NE 68106; Jeffrey D. Wells, PhD, FL International University, Dept Biological Sciences, 11200 SW 8th Street, Miami, FL 33199; and Leon G. Higley, PhD, University of Nebraska, 306B Biochemistry Hall, Lincoln, NE 68583-0760*

After attending this presentation, attendees will be aware of a method for calculating statistical confidence limits of an estimate of carrion insect age based on the categorical variable instar.

This presentation will impact the forensic science community by promoting the objective expression of the uncertainty associated with the typical forensic entomological conclusion and for a Postmortem Interval (PMI) estimated from any categorical measurement of decomposition.

The most common forensic entomological analysis involves estimating the age of a carrion insect associated with a corpse. If circumstances suggest that the individual insect was deposited on the victim following death (e.g., the victim was not colonized before death and the larva did not crawl to the victim after developing for some time on a different food source), this age value equals a minimum Postmortem Interval (PMImin). In the language of statistical models, an analyst "predicts" condition (e.g., specimen age) from response (e.g., specimen size), and a model of the relationship between condition and response also includes the effects of important covariates (e.g., insect species and environmental temperature).

It is desirable to be able to make an objective statement of uncertainty concerning any forensic science conclusion, and methods are available for calculating a confidence interval about an insect age estimate based on a continuous quantitative response(s) such as specimen length or weight; however, some forensic entomologists prefer to estimate age based only on life stage, because size can vary considerably between individuals of the same age and because size is also influenced by the specimen preservation method. Unfortunately, no statistical model has been proposed for estimating carrion insect age from a categorical response such as instar. This presentation will illustrate how statistical methods originally designed for estimating PMI from the categorical data of a carrion insect succession model can be applied to categorical insect development data.[1]

The *Lucilia sericata* (Diptera: Calliphoridae) development data recently published by Roe and Higley was used.[2] *L. sericata*, one of the most common and widely distributed insects used in death investigation, was reared at 11 constant temperatures from 12.5°C to 32.5°C (see reference 2 for a more complete description of the experimental methods and results). Age was converted to Accumulated Degree Hours (ADH) using threshold 10°C (ADH10) in order to increase the number of observations for an age category and to explore the utility of measuring time in ADH as a method for accommodating the well-known effect of temperature on development rate.

The analysis was confined to the life stages egg, first larva, second larva, and feeding third larval instar, because the post-feeding larva developed independently of temperature. Samples from different rearing temperatures were pooled into one set of age categories within which individual age in ADH10 varied by not more than 1%, and into an alternative set within which individual age in ADH10 varied by not more than 5%. Choice of this bin size involves a potential trade-off between age estimate confidence interval width (=precision) and lack of statistical power resulting from fewer individuals in an age class, which can yield a discontinuous confidence interval. Calculating a *p*-value, the criterion by which a potential specimen age is excluded, differed from the example illustrated for a succession model in that, as with all development models that have been proposed, an age estimate is based on a single specimen at a time rather than some set of observations.[1]

The age prediction performance of a model is indicated in part by the precision of an estimate and a continuous prediction interval. For this analysis, there was little difference in prediction performance between the model based on all temperatures compared to the model omitting the extreme high and low temperatures. The model based on 5% ADH10 bins performed slightly better than the model based on 1% ADH10 bins. For example, 95% prediction intervals in ADH units for the model with 5% ADH10 bins covering 15°C -30°C were: egg 100-505; first larva 195-755; second larva 560-1,040; and, third larva feeding 840-1,962.

This method is easy to implement in practice. It can be immediately applied to casework if a development model has been validated. It can be used to define the threshold for success in a validation study, and it also provides guidance for future development reference studies in that it specifies a minimum sample size needed for sufficient statistical power.

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Forensic Entomology, Postmortem Interval, Statistical Methods

H120 Thanatotranscriptome: Gene Expression in Cadaver Livers

Gulnaz T. Javan, PhD, Alabama State University, Forensic Science Program, 915 S Jackson Street, Montgomery, AL 36104; Ismail Can, BS, 915 S Jackson Street, Montgomery, AL 36104; Sheree J. Finley, MS, Alabama State University, 915 S Jackson Street, Montgomery, AL 36104; and Shivani Soni, PhD, Alabama State University, 915 S Jackson Street, Montgomery, AL 36104*

After attending this presentation, attendees will understand how to use the thanatotranscriptome to gain further insight into the pathways involved in the apoptotic machinery after death. Specifically, attendees will learn that RNA is stable in liver tissue of cadavers, and it is a suitable molecule for profiling gene expression even at longer periods of time after death up to 48 hours.

This presentation will impact the forensic science community by informing practitioners interested in using postmortem apoptosis patterns of select cadaver tissues with the goal of providing a method to estimate Postmortem Interval (PMI).

Gene expression investigations are a well-established part of antemortem studies with broad arrays of applications. Unfortunately, the study and implementation in the forensic fields are still in their infancy. The study of thanatotranscriptome apoptotic genes may prove useful relative to providing molecular evidence of active life after death. The goals of the study are to provide detailed insight into expression of 84 key genes involved in complex apoptotic pathways from liver tissues from actual, whole cadavers autopsied in forensic cases.

Tissue samples were removed by a medical examiner from the livers of four non-sequential victims of a heart attack caused by coronary heart disease and gunshot wounds. Complementary DNA (cDNA) was synthesized and the concentration was measured. To investigate the relationship between PMI and the expression levels of apoptosis, quantitative Polymerase Chain Reaction (PCR) arrays for specific apoptotic genes were performed. The PCR array contained the following five functional gene groups: (1) anti-apoptosis; (2) caspases and regulators; (3) death domain proteins; (4) induction apoptosis; and, (5) regulation of apoptosis. The apoptosis-related gene expression profile was assessed using human PCR arrays. The results show substantial down-regulation of the expression of anti-apoptotic functional gene groups when liver tissues from the control cadaver were compared with cases of increasing time of death. Significant anti-apoptotic genes such as *BAG3*, *BAK1*, *BAX*, *BIRC5*, *IL10*, *NAIP*, *NFKB1*, and *RIPK2* demonstrated expression reduced more than six-fold; however, gene expression levels of negative regulators of apoptosis such as *BCL10*, *BCL2L2*, *BCL2*, *CD40LG*, and *CIDEA* were also reduced. Additionally, the expression of death domain proteins such as *TNFRSF10A*, *TNFRSF11B*, *YNFRSF25*, *TNFRSF9*, and *TNFRSAF* was down-regulated more than three-fold. Certain genes responsible for the induction and positive regulation of apoptosis were greatly overexpressed. *ABL1*, *AIFM1*, *CIDEB*, *PYCARD*, and *TNFRSF10B* gene expressions increased greater than two-fold. *Caspases* and their regulators such as *CASP3*, *CASP4*, and *CASP9* gene expressions were also considerably up-regulated; however, the expression of the anti-apoptotic gene *XIAP* was also greater than 28-fold overexpressed.

This study was an analysis of the yield and apoptosis gene expression after a host dies as the interval of time since death increased in liver tissues of forensic cases. The results of this study demonstrate that optimum RNA extraction yields can be obtained even at a PMI of 48 hours. This result also suggests that RNA stability is viable for gene expression analysis from liver sample of cadaver cases.

In conclusion, this study shows RNA stability in liver postmortem samples, which makes it a suitable molecule for gene expression studies even at longer periods of time lapse up to 48 hours after death. The study design demonstrates a technique that will meet the demand for rapid and reproducible thanatotranscriptomic methods to correlate apoptotic gene expression patterns to establish a possible biomarker for estimating PMI.

Cadaver, RNA, Thanatotranscriptome

H121 An Evaluation of a New Rapid DNA Platform for Field-Forward Applications

Rachel E. Wiley, MFS, University of North Texas Health Science Center, Dept of Molecular and Medical Genetics, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107; Kelly Sage, BS, 4200 Bridgeview Drive, #1732, Fort Worth, TX 76109; Bruce Budowle, PhD, UNT Health Science Center, Forensic & Investigative Gen, 3500 Camp Bowie Boulevard, EAD 310, Fort Worth, TX 76107; and Bobby L. LaRue, Jr., PhD, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107*

The goal of this presentation is to educate attendees about the performance of a second-generation Rapid DNA genotyping platform.

This presentation will impact the forensic science community by reporting on an evaluation of the first (to this study's knowledge) fully automated second-generation Rapid DNA genotyping platform. This is impactful as the Federal Bureau of Investigation (FBI) has established new Quality Assurance Standards (QAS) guidelines for fully automated Rapid DNA genotyping platforms.

Utilization of a Rapid DNA platform to generate uploadable DNA profiles within and/or outside the traditional laboratory setting can be instrumental in improving workflow and reducing backlogs for DNA typing. The RapidHIT® ID system is a new second-generation Rapid DNA system that is configured to perform DNA extraction, Polymerase Chain Reaction (PCR) amplification, electrophoresis, and data analysis of reference swabs with an expert system to generate forensic DNA profiles comparable to traditional bench systems. The RapidHIT® ID system has a novel design that reduces its footprint and number of samples that must be run at any one time.

Reliable Short Tandem Repeat (STR) profiles from reference buccal swabs were obtained with nominal "hands-on" sample loading time and with a significant enhancement of workflow compared to the first-generation Rapid DNA systems. The RapidHIT® ID system was tested for reliability, concordance, reproducibility, and lack of contamination. Interpretation and sensitivity thresholds were determined, and even though the system is designed for reference buccal swabs, studies determining the effects of sample age, inhibitors, sample mixtures, and sample collection methods were performed. The new instrument provided results comparable with those from traditional DNA genotyping methodologies. Additionally, evaluation of the onboard expert system's capacity to generate fully automated STR profiles in accordance with the updated definition of Rapid DNA analysis as described in the December 2014 addendum to the FBI's QAS regarding Rapid DNA testing was performed.

Rapid DNA, Automated Genotyping, Arrestee STR Profiles

H122 Thanatophagy in Brain and Heart Tissues

Gulnaz T. Javan, PhD, Alabama State University, Forensic Science Program, 915 S Jackson Street, Montgomery, AL 36104; Insu Kwon, University of West Florida, 11000 University Parkway, Bldg 72, Pensacola, FL 32514; Sheree J. Finley, MS, Alabama State University, 915 S Jackson Street, Montgomery, AL 36104; and Youngil Lee, PhD, University of West Florida, 11000 University Parkway, Pensacola, FL 32514*

After attending this presentation, attendees will learn how thanatophagy occurs in a Postmortem Interval (PMI) -dependent manner in brain and heart tissues of heart attack victims. Specifically, attendees will learn what autophagy marker is actively expressed after a human dies.

This presentation will impact the forensic science community and practitioners interested in thanatophagy and its relationship to PMI determination and cadaver tissues.

Postmortem autophagy, also known as thanatophagy, commences after a human dies and endures as a function of the time of death. The progression of thanatophagy is a possible technique to estimate the time lapse since death. Approximately 30 various genes have been identified in mammalian autophagy. Among those gene products, Beclin-1, the mammalian orthologue of yeast Atg6, is a critical protein involved in the formation of autophagy-regulating macromolecular complex.

This study analyzed whether thanatophagy would increase proportionally compared with various postmortem intervals. Heart and brain tissues from four whole cadavers at different time frames of death were collected from autopsies of cardiac arrest victims in criminal cases. The tissues were analyzed using Western blot techniques and densitometry. First, the levels of LC3-I and LC3-II in cardiac tissues collected from different times of death, namely 6h, 16h, 36.5h, and 58h, were measured. It was found that thanatophagy occurred in a manner that correlated with the time of death from data demonstrating the levels of LC3 II expression along with other autophagy proteins: p62, BNIP3, Beclin-1, and Atg7. Next, the levels of an autophagy adaptor protein, p62, as an alternative marker of autophagy flux, which is co-degraded with LC3-II, were measured. Intriguingly, there was no observed decrease of p62 levels in either cardiac and brain tissues. Remarkably, BNIP3, a potent inducer of autophagy, was reduced as the time of death increased in the heart but was elevated in the brain; however, there was no expression of BNIP3 at 58h after death in either brain and cardiac tissues. Similar to LC3-II, both Beclin-1 and Atg7 increased as a function of time of death in cardiac tissues. Beclin-1 reached its maximum at 36.5h after death and continued expression until 58h after death.

This study suggests that although thanatophagy in the heart may occur in association with Beclin-1, brain thanatophagy appears separate from Beclin-1. Therefore, the current study reveals for the first time that thanatophagy occurs in the heart and the brain of cadavers in a manner dependent on the time lapse since death.

This study provides a potential insight into thanatophagy as a new method for precise determination of the time of death.

Thanatophagy, Cadaver, Postmortem Interval

H123 Novel Association Between the Thanatomicrobiome and Postmortem Interval (PMI)

Gulnaz T. Javan, PhD, Alabama State University, Forensic Science Program, 915 S Jackson Street, Montgomery, AL 36104; Ismail Can, BS, 915 S Jackson Street, Montgomery, AL 36104; Nathalie Lorenco, Alabama State University, 915 S Jackson Street, Montgomery, AL 36104; Sheree J. Finley, MS, Alabama State University, 915 S Jackson Street, Montgomery, AL 36104; and Jennifer G. Mulle, PhD, Emory University, 1518 Clifton Road, CNR 4053, Atlanta, GA 30322*

After attending this presentation, attendees will understand how to use high-throughput next generation sequencing analysis to characterize the thanatomicrobiome of internal organs from actual cadavers in criminal cases (e.g., homicide, suicide, and overdose). Specifically, attendees will learn methods to assess microbial diversity after death using PMI between 3.5 hours and 240 hours.

This presentation will impact the forensic science community by informing attendees interested in using high-throughput next generation sequencing of the microbial communities found in the internal organs, the oral cavity, and blood, if applicable, to develop a framework for determining PMI.

Accurate determination of the time of death, or PMI, is important in suspicious or unnatural deaths, particularly in criminal cases. The PMI provides critical information for crime scene reconstruction and in some cases can mean the difference between courtroom innocence or guilt. Conventional methods of determining PMI include rate (e.g., algor, livor, and rigor mortis) and concurrence methods (e.g., gastric emptying). These methods often do not provide definitive answers, which consequently leads forensic investigators to subjective conclusions. The thanatomicrobiome, or "microbes of death," is the diversity of microorganisms involved in human decomposition.

This study hypothesized that as a human body decays, time-dependent changes in the thanatomicrobiome within different body sites will be more predictive of the time of death. The objectives were to analyze the thanatomicrobiome of internal organs (brain, heart, liver, and spleen), the mouth cavity, and blood of human cadavers. To assess this hypothesis, a cross-sectional study was performed by sampling 28 human corpses with PMIs between 3.5 hours and 240 hours. The samples were obtained from between one to six body sites. 16S ribosomal RNA (rRNA) next-generation sequencing and analysis revealed characteristic time-dependent changes in the thanatomicrobiome community composition that were associated with time of death. Diversity was examined from two perspectives: (1) the overall richness (i.e., the number of distinct microorganisms found within the microbiome); and, (2) the Shannon Diversity (i.e., both richness and evenness, the distribution of abundance among distinct taxa). The diversity was expressed as the number of Operational Taxonomic Units (OTUs) and was quantified using the Chao1 richness estimator. Measures of diversity were screened for group differences using a statistical Analysis of Variance (ANOVA). The thanatomicrobiome results revealed the relative abundance of the 20 most dominant genera in all samples that formulate the diversity of the microorganisms most responsible for decomposition. Remarkably, the obligate anaerobe, *Clostridium*, was predominant in all cadavers in all samples except the oral cavity.

To date, relatively few studies have investigated the microbiome of whole cadavers from actual criminal cases in a forensic context. These results suggest that the thanatomicrobiome could be useful to criminal investigators and forensic scientists as a new source of data for identifying PMI.

Thanatomicrobiome, Cadaver, Postmortem Interval

H124 Inferring Patterns of Occupancy From Human Microbial Signatures

Simon Lax, 5128 S Kimbark, #3NW, Chicago, IL 60615; and Jack Gilbert, PhD, University of Chicago, Dept of Ecology and Evolution, 1101 E 57th Street, Chicago, IL 60637*

After attending this presentation, attendees will better understand current methods used to match humans to places they have inhabited and objects they have interacted with through shared microbial signatures. This presentation will educate attendees about the extent to which the skin microbial community of occupants shapes the microbial ecology of built environments.

This presentation will impact the forensic science community by incorporating modern methods in microbial ecology and bioinformatics into forensic applications and developing the concept of human microbial fingerprints.

This presentation will discuss three recent large-scale studies of temporal microbial interaction between humans and the built environment focusing on: (1) personal homes; (2) on hospital rooms; and, (3) on the shifting microbial signatures on shoes that accompanies a change in location.

Human skin and respiratory cavities harbor a vast array of microbial consortia that can be readily dislodged and transferred to their surrounding environment. Such communities are extremely diverse, with great variation both between individuals and between different body sites of the same individual, yet they are sufficiently stable over time that interpersonal variation exceeds temporal variation, even across sampling intervals of many months. The idea that individuals harbor a unique "*microbial fingerprint*" has been well supported by the vast number of microbial surveys that have accompanied the rise of high-throughput sequencing technology, and such "fingerprints" have been shown to have great predictive power in both medical diagnostics and the emerging field of microbial forensics, which utilizes microbial signatures to determine interactions between individuals and objects.

In this study of home-associate microbial communities, residents were successfully matched to their homes based on similarity between the microbial communities of their skin and home surfaces. Further, the relative contribution of different residents, including pets, to different surface types was also able to be determined. This study was even able to predict when a resident of a home had left for travel by determining when their microbial signal diminished or vanished.

In a second ongoing study of hospital rooms, the methods developed for the home study are being applied to a system in which the resident of the environment is constantly changing. The unique features of hospital rooms, such as their intensive cleaning and highly regulated temperature and humidity, allowed for testing of a number of hypothesis regarding the strength and persistence of human microbial signatures in built environments.

Finally, the results of a study on the forensic applications of shoe-associated microbes in determining where someone has recently walked will be presented. In this study, it was found that supervised learning techniques could easily distinguish which of two study participants a shoe sample was taken from, and that models trained on floor samples alone were able to correctly identify which participant a shoe sample was taken from. Major shifts in shoe-associated microbiota with a change in environment were also able to be detected and correlation in shoe and floor microbial communities could be used to match the study participant to their location at different time points. This same study also collected shoe and phone samples from 89 different participants at three different scientific conferences and was largely able to match participants from individual conferences based on microbial similarity, suggesting widespread microbial interaction between individuals temporarily inhabiting the same space.

These three studies represent some of the largest efforts yet to employ novel high-throughput sequencing methods to microbial forensics in built environments. This presentation will elucidate the extent to which current methods are able to match individuals to spaces they have inhabited and will discuss both the limitations and potential of these methods to impact forensic science.

Trace Evidence, Forensic Microbiology, Molecular Forensics

H125 Evaluating the Skin Microbiome as Trace Evidence on Common Surface Types

Jessica L. Metcalf, PhD, University of Colorado, Boulder, Dept of Ecology and Evolution, Boulder, CO 80309-0334; Embriette R. Hyde, BS, Baylor College of Medicine, Dept of Molec Virology & Microbiology, Houston, TX 77030; Se Jin Song, BA, University of Colorado, Dept Ecology & Evolut Biology, Boulder, CO 80309; Simon Lax, 5128 S Kimbark, #3NW, Chicago, IL 60615; Jack Gilbert, PhD, University of Chicago, Dept of Ecology and Evolution, 1101 E 57th Street, Chicago, IL 60637; David O. Carter, PhD, Chaminade University of Honolulu, Forensic Sciences Unit, Honolulu, HI 96816; and Rob Knight, PhD, University of California San Diego, Dept of Pediatrics, 9500 Gilman, La Jolla, CA 92093-0763*

After attending this presentation, attendees will understand the reproducibility of hand microbial communities that are transferred to multiple surface types. Attendees will be presented with results from recent skin transfer experiments in which it was determined whether or not surface type (metal, wood, ceramic, glass, and plastic) affects the ability to transfer a person's unique skin microbial signature to a surface.

This presentation will impact the forensic science community by revealing basic transfer properties of an individual's unique skin microbial community to common surface materials, which has potential as trace evidence for criminal investigators.

The skin microbiome is highly individual, to the extent that two people's hands can differ in more than 80% of the types of microbes found. A previous study determined that people's personalized microbial communities are transferred to objects, such as computer keyboards and surfaces in the physical spaces people inhabit. Importantly, the composition of a person's skin microbial community appeared generally stable over time. As a result, skin microorganisms have great potential to serve as trace evidence, and they are uniquely positioned to augment friction ridge comparison when sufficient ridge detail is not available to make a positive identification. Thus, the potential for microorganisms to reveal whether a particular person has touched an object or has recently been in a space is substantial.

This study hypothesized that some surface types will maintain a person's skin microbial signature better than others. It is well known among forensic scientists that friction ridges are harder to recover off some types of surfaces than others. In a similar manner, it is hypothesized that surface types may vary in their ability to receive or retain skin microbial communities after contact. To test this hypothesis, skin transfer experiments were carried out at the University of California San Diego (UCSD). To characterize each participant's unique skin microbial signature, skin from each participant's hand was swabbed for five days leading up to the experiment. The ability for skin microbes to transfer to metal, wood, ceramic, glass, and plastic tiles was tested by swabbing sets of tiles immediately after they were touched. Each participant in the experiment touched five tiles of each surface type. Each tile was only touched one time. Additionally, untouched control tiles of each surface type were swabbed.

All swabs were stored at -20°C until DNA extraction using the MoBio PowerSoil DNA isolation kit following the Earth Microbiome Project (EMP) standard protocols. 16S ribosomal RNA (rRNA) Polymerase Chain Reaction (PCR) amplicons were generated from each sample, multiplexed, and sequenced on the Illumina® HiSeq™ 2500 platform at UCSD. Sequence data were processed and analyzed using the Quantitative Insights into Microbial Ecology (QIIME) bioinformatics open-source software. Resulting sequences were classified into Operational Taxonomic Units (OTU) and identified to known taxonomy using the Greengenes reference database. Phylogenetic metrics were used to estimate alpha diversity and beta diversity. Supervised learning and Bayesian source-tracking methods were used to estimate the ability to accurately link surface microbial communities with a participant in the experiment. Microbial communities recovered from tiles were compared to those sampled from each participant's hand previous to the experiment as well as to untouched control tiles. Results will be presented.

The results provide a robust basis for understanding whether microbial trace evidence has potential for crime scene investigation.

Skin Microbiome, Trace Evidence, Bacteria

H126 Drugs and Bugs (Bacteria): Does What You Use Relate to What You Grow?

Jennifer L. Pechal, PhD, Michigan State University, 243 Natural Science Bldg, East Lansing, MI 48824; Carl J. Schmidt, MD, Wayne County MEO, 1300 Warren, Detroit, MI 48207; Heather R. Jordan, PhD, Mississippi State University, PO Box GY, Mississippi State, MS 39762; and M. Eric Benbow, PhD, Michigan State University, Depts of Entomology & Osteopathic Med Specialties, 288 Farm Lane, East Lansing, MI 48824*

After attending this presentation, attendees will understand how drug use impacts the human postmortem microbiome. While several studies have documented shifts in microbial community composition and abundance from various locations on a body during the decomposition process, much less is understood about how the postmortem microbiome varies among groups of individuals that have a history of substance use/abuse or whose death results from it. This presentation will include data from the largest set of postmortem microbiome human remains, which have been swabbed from six locations on each body, providing insight into how drug use, such as hydrocodone, cocaine, opiates, or heroin, may potentially be associated with the postmortem microbiome and perhaps be an indicator of antemortem interactions of the microbiome and lifestyle.

This presentation will impact the forensic science community by providing the only database to date of the human postmortem microbial communities found on human cadavers resulting from drug-related deaths. Researchers have typically focused on the potential effects of drugs on the microbiome of living individuals in pharmaceutical development for personalized health care or the impacts of illicit drug use on perinatal health and mortality; however, there has yet to be a study characterizing the postmortem microbial communities of individuals with substance abuse. This presentation will add to the research targeted on identifying microbial communities after death and provide real-world data for advancing the field of forensic microbiology with the ultimate goal of increasing the potential to routinely use microbial communities in death investigations.

Microorganisms are ubiquitous in the environment and associated with humans both antemortem and postmortem, but are often overlooked and underutilized biological indicators of circumstances and length of time since death. Due to the ability to culture only a small subset of currently known microbes (e.g., pathogenic bacteria), they have received little research attention for their potential use in forensic sciences. There is a paucity of data available describing the postmortem microbiology and microorganism biodiversity occurring on human cadavers, particularly on the naturally occurring variation of indigenous microflora residing on or in the human body during decomposition; however, recent work studying decomposition of model organisms (e.g., swine and mouse carcasses) suggests that microbial communities are quite dynamic during the postmortem interval. The goal of this study was to describe the human postmortem human microbiome associated with individuals in a major metropolitan city (Detroit, MI) whose deaths resulted from a variety of drug usages.

Microbial samples were collected from human remains received into the Wayne County Medical Examiner's Office in Detroit, MI. Samples were collected from 40 human cadavers (male and female) representing different causes of death (e.g., drug type). DNA-free (sterile) cotton-tipped swabs were used to aseptically collect individual microbial communities from six anatomic locations: the external auditory canal, nose, mouth, umbilicus, rectum, and the trabecular space between the inner and outer tables of the occipital bone. DNA was extracted using a modified protocol of a commercially available kit; all DNA was quantified to ensure quality samples for metagenomic sequencing. The 16S ribosomal RNA (rRNA) V4 gene region was sequenced for each sample using a 2x250bp paired-end approach using a high-throughput metagenomic sequencing platform. The microbial community composition of cadavers resulting from drug use was statistically significantly different ($P < 0.05$) from those microbial communities detected on individuals that died from natural disease (cardiovascular in origin). Additionally, there were distinct microbial communities among the sets of remains that varied based on sex and anatomic region from which the sample was obtained; however, the differences of microbe communities were not as distinct among comparisons of the various types of drugs present in the individual. Overall, these data demonstrate that the postmortem human microbiome is different in deaths due to drug use, although how drug use affects the living microbiome is also unknown at this time and may affect the resulting postmortem microbiome.

In conclusion, this study contributes a unique data set to previous research of the postmortem microbiome; specifically, partnering with a medical examiner's office allowed the opportunity to characterize microbial communities associated with deaths due to drug use. These data provide striking evidence that drug use influences the postmortem microbial community of an individual, and future work with additional partnerships with medical examiners' offices should be considered to expand upon the current data set. It also suggests that the microbiome in the living may be different in drug users compared to the rest of the population: such a relationship could prove valuable in future forensic contexts.

Postmortem Microbiome, Drug Use, Medical Examiners

H127 A Predictive Knowledgebase Linking Microbial Signatures to Human Lifestyle Characteristics

Jack Gilbert, PhD, University of Chicago, Dept of Ecology and Evolution, 1101 E 57th Street, Chicago, IL 60637; Jose Lopez, PhD, Nova Southeastern University, Oceanographic Center, Nova Southeastern University, 8000 N Ocean Drive, Dania Beach, FL 33004; Simon Lax, 5128 S Kimbark, #3NW, Chicago, IL 60615; George T. Duncan, PhD, Broward County Crime Lab, 201 SE 6th Street, Rm 1799, Fort Lauderdale, FL 33301; and Jessica L. Metcalf, PhD, Chemistry and Biochemistry, Jennie Smoly Caruthers Biotech Bldg, Boulder, CO 80309*

After attending this presentation, attendees will understand recent advances in the microbial ecology of skin-associated trace evidence and why it may be useful for predicting human lifestyle characteristics. Attendees will be presented with results from recent experiments in which microbial communities were sampled from mock crime scenes and compared against a nascent database to determine if human characteristics of the occupants can be determined.

This presentation will impact the forensic science community by revealing the potential for Next Generation Sequencing (NGS) of microbial communities associated with the human occupants of a space to be used in trace evidence.

The composition of microbial organisms associated with skin is unique to an individual. This is because the experiences each person has since birth are unique, and these physical interactions with the world are what allow microbes to colonize and form communities ("microbiomes") on our bodies. Even identical twins, whose microbiota are significantly more similar than other siblings, each have a unique profile. Growing up together means that one will share similar microbial sources, but the key to forensic application of the microbiome is in the differences. Each person is born sterile and is normally first colonized by bacteria associated with the mother. Subsequent to that, the microbial assemblages are shaped by, for example, what people eat, whom people touch, where people live, and how much time people spend indoors versus outdoors.

While an individual's core microbiome is considered stable by the age of two to three years old, it can still undergo variation as a person changes aspects of their lifestyle that causes them to be exposed to different microbial worlds. The skin microbiome is the primary interface with the world and the interface one most readily leaves behind when he/she interacts with a space. The microbial communities on the hands, noses, buttocks, and feet are unique to each person, but are also impacted by lifestyle and physical interactions with others. This study therefore posits that the microbiome can be highly predictive of elements of one's self and one's lifestyle. Therefore, there is a huge untapped demand in the criminal justice community for "class" information to be used for investigations. This gap could be filled in part by using the microbiome of human skin.

When more laboratories have the use of NGS instrumentation at their disposal, the likelihood that microbiome data could fill this gap increases. Since forensics is an application science, human microbiome profiles may be very important in many ways to the investigator. In a recent study, individual families were matched to their homes even when they had moved to another residence. This is based on a comparison of individuals to their environment, but also and more importantly, their microbiota transferred to a new residence where they had moved. A recent true scenario that occurred in a South Florida environment is presented as an example of the possible usefulness of this analysis. A man had begun to cohabitate with an individual of the same gender, but of a significantly different age. The man was found murdered in this apartment and the younger cohabitant was known to leave the house and return later in the day on a routine basis. A conventional DNA analysis of the handle of a knife as well as many other items, which the temporary occupant may have touched, yielded no profile. The questions now arose: Who was this person? What were his traits? More importantly for the investigator in this case, where did this individual possibly live or work?

To date, the evidence to support this idea has been limited by small studies and anecdotal enquiry. A systematic analysis of a human population is being performed around Miami, FL, to determine categorically whether elements of their lives can be predicted from their microbiome, both on their bodies and that left behind on surfaces with which they interacted. In doing so, a list will be created of highly specific microbial biomarkers for particular traits (e.g., young adult female vegetarian, who lives in the suburbs, and works in a bakery or bread counter). A sophisticated artificial neural network and database will also be built to enable extrapolation of microbial signature detection to other samples, so that a person's traits can be detected from the microbial community they leave behind. This proof of principle study will form the foundation of a forensic effort in Miami, FL, to create a new suite of trace evidence options that can be leveraged by investigators to help shape their interpretation of a crime scene.

Trace Evidence, Forensic Anthropology, Microbiome

H128 Do Postmortem Skin Microbial Communities Change During Morgue Transit and Cooler Storage?

Whitney A. Kodama, BA, Chaminade University of Honolulu, Forensic Sciences Unit, 3140 Waialae Avenue, Honolulu, HI 96816; David O. Carter, PhD, Chaminade University of Honolulu, Forensic Sciences Unit, Honolulu, HI 96816; Jessica L. Metcalf, PhD, Chemistry and Biochemistry, Jennie Smoly Caruthers Biotech Bldg, Boulder, CO 80309; and Rob Knight, PhD, University of California San Diego, Dept of Pediatrics, 9500 Gilman, La Jolla, CA 92093-0763*

After attending this presentation, attendees will understand the impact of morgue storage and handling of a decedent on postmortem skin microbial communities.

This presentation will impact the forensic science community by identifying an optimal timeframe during which to collect microbial evidence from skin.

Microbial communities change throughout the decomposition process. Because these changes are predictable, microbial communities have the potential to be used to estimate Postmortem Interval (PMI); however, there are many factors affecting microorganisms that must still be researched, including cause of death, antibiotic/drug use, environment, and others. One unexplored variable that is relevant to most death investigations is the storage and handling of the decedent prior to autopsy. This process includes transport to the morgue, general intake procedures, and cooler storage leading up to autopsy.

It is important to learn how morgue storage and handling of the decedent prior to autopsy affects skin microbial communities because it could affect the estimation of PMI and also the potential use of microbes as trace evidence. Individual skin microbial communities are personalized and relatively stable over time. The forensic value of these communities is that they are transferred to locations and objects with which a person interacts/contacts. Therefore, individuals can be associated with objects and locations. Establishing a timeline of skin microbial community change, if there is any, during morgue transit and cooler storage can provide valuable information to a death investigation because it has the potential to provide an optimal timeframe during which to collect microbial evidence from the skin of the deceased. Furthermore, skin microbial communities can corroborate other forms of trace evidence and fingerprint analysis and also have the potential to provide a positive identification when traditional forms of evidence are either unavailable or provide insufficient information.

To investigate the effect of storage and handling on the skin microbial community after death, ten death scenes under the jurisdiction of the City and County of Honolulu Department of the Medical Examiner were attended. Both hands of the deceased were swabbed upon arrival. Objects (e.g., door knobs, light switches, phones) that the deceased commonly touched were also swabbed. Once at the morgue, both hands were swabbed at six-hour intervals until an autopsy was conducted or the body was released following external examination. To establish the microbiome of the morgue environment, samples were also collected from transport surfaces, the inside of new and used body bags, morgue cooler surfaces, and the autopsy room surfaces.

All swabs were stored at -20°C until analysis. DNA was extracted from the swabs using the PowerSoil® DNA isolation kit and stored at -20°C. 16S small subunit ribosomal RNA (rRNA) genes were used to characterize bacterial communities. Polymerase Chain Reaction (PCR) amplicons were combined from each sample and sequenced on the Illumina® HiSeq™ 2500 platform. Sequence data were processed and analyzed using the bioinformatics pipeline available in the Quantitative Insights into Microbial Ecology (QIIME) open-source software. Resulting sequences were filtered to remove low-quality sequences, classified into Operational Taxonomic Units (OUT) and identified to known taxonomy through the Greengenes reference database. QIIME and the phylogenetic metric UniFrac were used to estimate alpha diversity and beta diversity. Supervised learning and Bayesian source-tracking methods were used to estimate the ability to accurately link microbial samples with a deceased person.

These data were used to test this study's null hypothesis that skin microbial communities will not change during storage and handling of the deceased. Results will be presented.

Taphonomy, Bacteria, Microbiome

H129 The Human Postmortem Microbiome and Manner of Death

M. Eric Benbow, PhD, Michigan State University, Depts of Entomology & Osteopathic Med Specialties, 288 Farm Lane, East Lansing, MI 48824; Jennifer L. Pechal, PhD, Michigan State University, 243 Natural Science Bldg, East Lansing, MI 48824; Carl J. Schmidt, MD, Wayne County MEO, 1300 Warren, Detroit, MI 48207; and Heather R. Jordan, PhD, Mississippi State University, PO Box GY, Mississippi State, MS 39762*

After attending this presentation, attendees will understand how the human postmortem microbiome varies among individuals and if the manner of death affects these microbial communities. In addition, attendees will have a better appreciation for the challenges associated with the potential use of the postmortem microbiome in forensic investigations. While several studies have demonstrated that the process of describing changes in microbial communities during the decomposition process of vertebrate remains has the potential for use in estimating the minimum Postmortem Interval (PMI_{min}) range, it is less understood how the postmortem microbiome varies among individuals in general and if the circumstances of death affect these communities. This presentation will include data from the largest set of human remains that have been swabbed from six places on each body, providing information on the human microbiome from real-world death investigations.

This presentation will impact the forensic science community by providing the largest database to date of the human postmortem bacterial communities found on human cadavers during routine death investigations at a medical examiner's office. Determining the PMI_{min} can be a critical process following homicides or unwitnessed deaths, and resolving the precise window of time and location of both the decedent and witness(es) is then essential for the investigative process. Because microorganisms are ubiquitous in the environment but difficult to characterize, they have historically received little research attention for their potential use in forensics, especially in such activities as estimating a PMI_{min} range. Further, little is known about postmortem microbiology and biodiversity in human cadavers, particularly the microbial heterogeneity of indigenous microflora residing on or in the human body throughout decomposition or related to the manner of death; however, recent work suggests that the healthy human microbiome is quite dynamic in both space (different parts of body) and time (community changes on or in a person). One of the first steps in evaluating the true potential of the postmortem human microbiome in forensics is to establish a baseline database of known bacterial taxa found on cadavers resulting from various manners of death and in differing progressions of the decomposition process. The goal of this study was to describe the human postmortem microbiome swabbed from different anatomic regions and assess patterns in relation to manner of death and autopsy-estimated PMIs from routine cases in a major metropolitan city — Detroit, MI. In this presentation, the largest baseline database of the postmortem microbiome developed from human remains investigated by a medical examiner's office is presented.

Bacterial samples were collected from human remains received into the Wayne County Medical Examiner's Office in Detroit, MI; each cadaver represented different circumstances of death and progression of decomposition. Individual DNA-free sterile cotton-tipped swabs were used to aseptically collect individual bacterial communities from six body areas: the external auditory canal, nose, mouth, umbilicus, rectum and the trabecular space between the inner and outer tables of the occipital bone. DNA extractions were performed using standard kits and quantified. All bacterial DNA samples were sequenced using a 2x250bp paired-end approach. Library construction and sequencing of the 16S ribosomal RNA (rRNA) V4 gene region was performed by the Michigan State University Genomics Core Facility using a modified version of the protocol adapted for high-throughput metagenomic sequencing. Samples were collected from 100 human cadavers representing the following manners of death: homicide, suicide, accident, and natural. There were distinct microbiomes among the sets of remains that varied based on sex and time of death; however, microbiomes were most different among the areas of the body that were swabbed compared to overall variation among individual microbiomes, suggesting additional research for evaluating the potential use of these communities in forensic investigations.

This project greatly expands previous research of the postmortem microbiome by partnering with a medical examiner's office to characterize bacterial communities associated with human cadavers during routine death investigation. These data offer a transformative way to solve common practical issues associated with studying human decomposition while moving the science of the human postmortem microbiome in a direction that addresses the importance of replication and understanding the initial variability in real-world cases.

Postmortem Microbiome, Medical Examiners, Death Scene Investigation

H130 Development of a Free, Customizable, Forensic Autopsy Report Generator

Matthew D. Cain, MD, University of Alabama at Birmingham, 619 S 19th Street, Birmingham, AL 35233-7331; Yihong R. Ma, MD, University of Alabama at Birmingham, 619 S 19th Street, Birmingham, AL 35233-7331; and Daniel W. Dye, MD, Jefferson County Coroner/Medical Examiner Office, 1515 6th Avenue, S, Rm 220, Birmingham, AL 35233*

After attending this presentation, attendees will understand the potential utility of a forensic autopsy report generator, available at no cost, that has been developed.

This presentation will impact the forensic science community by providing a free webpage to Medical Examiners (MEs) and coroners to facilitate standardized autopsy reports. Users of this software have the option to copy the webpage and customize the templates according to their office's format.

Autopsy reports provide important insights into cause and manner of death of an individual. Families, lawyers, and other medical professionals read these reports; therefore, it is important to generate both clear and accurate accounts of autopsy findings. These cases are often used in court, so it is crucial to keep errors to a minimum. Pathologists strive to meet these standards of excellence; however, workloads often dictate expedient work to achieve appropriate deadlines. To assist in this dilemma, this study presents work on an autopsy report generator.

The report generator is a webpage designed using Hypertext Markup Language (HTML), JavaScript™ (a browser-based programming language), and Twitter® Bootstrap. HTML is the standard language used to generate a webpage.[1] JavaScript™, a commonly used programming language for web development, was utilized to impart functionality to the webpage.[2] The ability of the webpage to interpret user data, incorporate inputs into a paragraph, and generate a report was made possible by JavaScript™. Twitter® Bootstrap is an open source set of instructions and makes the webpage aesthetically pleasing, user-friendly, and compatible with mobile devices.[3] The webpage is hosted at no cost on GitHub.[4] This was chosen so users could "fork" the webpage, or copy it. By "forking" the webpage, users can modify the report templates that are held within the JavaScript™ files. These modifications will be reflected in the new web address that is generated after copying the webpage. To assess the utility of this resource, the time required to dictate a case versus using the generator was tracked. Editing time was also evaluated and compared using t-tests.

A webpage was successfully designed that generates forensic autopsy reports in full paragraph format. To evaluate efficiency, this study recorded times for comparison against conventional dictation. Overall, the average time to dictate a case was 673 seconds versus 276 seconds using the report generator ($p = 0.04$). Editing time was significantly reduced as well (>11 minute reduction, $p = 0.046$). Several gunshot cases were tested in the webpage and had minimal editing requirements afterward since the webpage has a function to help organize an "Evidence of Injury" section. The webpage also helped ensure accuracy by checking for consistent features, such as gender, throughout the report. Cases with deviations from the normal template, such as a gunshot wound through the liver, automatically applied the user-defined description, inserted the appropriate weight, and removed normal details, such as "the capsule is intact."

The autopsy report generator created accurate and consistent reports while dramatically reducing the time required by the ME to both generate and edit a report. While it was not feasible to track the number of edits required by dictation versus the webpage, it is believed that the webpage had less because of the difference in editing times. Because the software's user interface implements Twitter® Bootstrap, tablet devices are readily compatible with the webpage. To improve efficiency, physicians could employ tablet devices in the morgue, enter values and notes at the time of autopsy, and have a fully prepared report at the end of the case.

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Forensic Autopsy, Report, Generator

H131 Detection and Differentiation of Early Acute and Following Age Stages of Myocardial Ischemia With Quantitative Postmortem Cardiac Magnetic Resonance (PMCMR)

Wolf-Dieter Zech, MD, University of Bern, Institute of Forensic Medicine, Dept of Forensic Medicine and Imaging, Bühlstrasse 20, Bern 3012, SWITZERLAND; Nicole Schwendener, HF, Institute of Forensic Medicine, University of Bern, Bühlstr. 20, Bern 3012, SWITZERLAND; Anders Persson, MD, PhD, CMIV, Linköpings Universitet/US, Linköping 581 85, SWEDEN; Marcel Warntjes, PhD, CMIV, Linköping University, Linköping, SWITZERLAND; Frederick Schuster, MD, Institute of Forensic Medicine, Buehlstrasse 20, Bern, SWITZERLAND; Fabiano Riva, PhD, Institute of Forensic Medicine Bern, Buehlstrasse 20, Bern, SWITZERLAND; and Christian Jackowski, MD, EMBA, Institute of Forensic Medicine, University of Bern, Bühlstr. 20, Bern, Canton Bern, SWITZERLAND*

After attending this presentation, attendees will better understand MR quantification, which is used as a new and promising tool in postmortem cardiac imaging.

This presentation will impact the forensic science community by presenting the conclusion that PMCMR quantification is feasible for characterization and differentiation of early acute and following age stages of myocardial infarction based on quantitative values. Quantitative PMCMR enables assessment of early acute myocardial ischemia, which is not visible in conventional PMCMR or heart dissection.

In the last decade, PMCMR has been introduced and established in the field of postmortem forensic imaging. PMCMR can be used as a valuable adjunct to forensic autopsy in assessing cardiac pathologies and cardiac-related deaths.[1-5] Recently, quantitative MR sequences have started to be used in PMCMR. Quantitative MR data allow for an objective characterization and differentiation of healthy tissues and pathologic tissues based on quantitative values such as T1 and T2 relaxation times.[6-8] Thus far, no quantitative values for the different histopathological age stages of myocardial infarction have been established in postmortem 1.5T MR imaging.

The goal of the present study was to assess quantitative T1 (in ms), T2 (in ms), and Proton Density (PD) (as %) values of early acute and following age stages of myocardial ischemia and to correlate these values to their corresponding histologic findings.

In 80 deceased individuals (25 female, 55 male) from forensic cases, a cardiac MR quantification sequence was performed prior to cardiac dissection. Ischemic myocardial lesions and unremarkable myocardium were MR quantified and correlated with histology.

Seventy-three myocardial lesions were detected by PMCMR. These lesions were characterized histologically as early acute (n=39), acute (n=14), subacute (n=10), and chronic (n=10) ischemic lesions. Further early acute ischemic lesions (n=22) not visible at heart dissection were detected at routine histological heart examinations and showed no visible signal alterations in conventional PMCMR. All detected lesions (n=95) were MR quantified. Statistical analysis revealed that based on their quantitative T1, T2, and PD values, a significant difference between all tested age groups of myocardial ischemia (early acute, acute, subacute, and chronic ischemic lesions) can be determined among one another and between normal myocardium.

The results of this study provide a basis for computer-aided diagnoses of myocardial ischemia in postmortem cardiac magnetic resonance.

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Quantitative Postmortem MRI, Myocardial Ischemia, Forensic Imaging

H132 Forensic Radiology Pitfalls

Mark A. Giffen, Jr., DO, 101 E 6th Street, Apt 501, Winston Salem, NC 27101; Jerri McLemore, MD, Wake Forest School of Medicine, Dept of Pathology, Medical Center Boulevard, Winston-Salem, NC 27157; and Jason Powell, MD*, Veterans Administration, 1705 Gardner Drive, Wilmington, NC 28405*

The goals of this presentation are to: (1) educate attendees as to basic Computed Tomography (CT) radiology principles; and, (2) highlight important imaging artifacts using three case studies of firearm injuries to the head. After attending this presentation, attendees will better understand CT radiology used in a forensic setting and some of the pitfalls associated with the interpretation of antemortem and postmortem acquisition of images.

This presentation will impact the forensic science community by discussing differences that may occur between images obtained during life versus those obtained in the postmortem interval using three examples of firearm injuries of the head. As the field of radiology is an entire discipline unto itself, this presentation will merely highlight some of the fundamental principles of radiology (especially CT radiology) as they apply to forensic investigation.

CT imaging is becoming more attractive as a supplemental tool for autopsy to coroner's/medical examiner's offices of all sizes throughout the country; therefore, forensic pathologists are increasingly interpreting CT images obtained prior to the decedent's death or, in fewer offices, obtained after death to guide the autopsy. This trend will only increase as clinician's reliance on CT imaging surpasses the use of conventional X-rays. Though forensic radiology is becoming an established field of study, most forensic pathologists do not have the volume of imaging or resources necessary to support the use of a full-time forensic radiologist. Many municipalities are therefore expected to perform these radiologic examinations and interpretations on their own. This growing practice can prove difficult as most forensic pathologists have only a basic knowledge of CT radiology and basic understanding of artifactual findings. Difficulties can emerge when antemortem CT images are available but without the reported interpretation, especially when artifacts commonly known to radiologists but not to forensic pathologists are present. Three examples of short-term survivors who had firearm injuries of the head and who underwent antemortem CT imaging are discussed. Review of copies of the antemortem CT images of the heads that were available to the forensic pathologist prior to the start of the autopsies showed radiodense objects suggestive of pellets from a shotgun, which was not consistent with the gross appearance of the injuries. The apparent discrepancies were resolved based on comparisons with the conventional X-rays and an understanding of the basic differences in antemortem and postmortem acquisition of CT images.

To explain these differences, a brief overview of CT radiologic theory will be presented with a discussion of how CT images are obtained as well as a basic overview of computer-aided reconstruction techniques. The manipulation of CT images using window and level adjustment to clarify images and decrease artifacts will also be highlighted.

Computed Tomography, Artifact, Forensic Investigation

H133 Postmortem Computed Tomography (PMCT) and Initial Experiences in Postmortem Angiography in Pediatric Cases

Silke Grabherr, PhD, Centre Universitaire Romand de Médecine Légale, Chemin de la Vulliette 4, Lausanne 25 1000, SWITZERLAND; Christine Chevallier, CURML, Bugnon 21, Lausane, Vaud 1011, SWITZERLAND; Beatriz V. Krentz, Department of Pathology, Faculdade de Medicina da, Av. Dr Arnaldo 455, CEP 1246-903, São Paulo, BRAZIL; Leonor T. Alamo, Department of Diagnostic and Interventional Radiol, Rue du Bugnon 46, Lausanne 1011, SWITZERLAND; Coraline Egger, MD, University Center of Legal Medicine, Rue Michel-Servet 1, Genève 1211, SWITZERLAND; and Jochen Grimm, MD, JD, Rue du Bugnon 21, University Center of Legal, Medicine Lausanne, Lausanne, VD, SWITZERLAND*

After attending this presentation, attendees will understand the role of PMCT in investigating the cause of death in pediatric cases, especially when compared to conventional autopsy. Attendees will also better understand the newest ongoing research and initial experiences in the field of PMCT-angiography.

This presentation will impact the forensic science community by demonstrating the advantages and limitations of PMCT for investigating pediatric cases and explain why modern research in PMCT-angiography may increase the quality of the postmortem examination.

Performing a postmortem Multidetector Computed Tomography (MDCT) scan has already become routine in some forensic institutes, especially in Switzerland. In order to also investigate the vascular system, different techniques of PMCT-angiography have been tested. Such radiological techniques also play an increasing role in in-hospital deaths where consent of next of kin is needed to perform autopsy. Such consent is often difficult to obtain in cases of deceased children. Consequently, radiological methods could be an alternative to investigate the cause of death.

In order to define the performance of PMCT compared to conventional autopsy in children, a retrospective study was performed at a medical center in Switzerland on a group of 26 children aged 0 years to 12 years old who received both conventional autopsy and PMCT. All reported findings were extracted from radiological and autopsy reports and compared to each other. All findings were grouped according to their importance for the final diagnosis of the cause of death and to the anatomical structure in which they were found (organs, vascular system, soft tissue, and skeletal system).

Overall, a significantly larger number of findings were detected at autopsy compared to PMCT. Autopsy proved to be superior to PMCT notably at detecting organ, soft tissue, and vascular findings, while PMCT was superior in detecting bone findings; however, for findings essential to define the cause of death, no statistically significant difference between these methods was found.

The results of this study led to the conclusion that PMCT is less sensitive for detecting findings than conventional autopsy; however, essential findings can mostly be seen with both methods and PMCT is superior to autopsy for detection of bone lesions in the postmortem investigation of children.

In order to increase the quality of the radiological exam, contrast agent can be applied that enhances soft tissue and organs and allows the investigation of the vascular system. In adult postmortem imaging, this leads to a significant increase of the sensitivity of the exam. The most widespread technique of postmortem angiography today is the so called Multi-Phase Postmortem CT-Angiography (MPMCTA). Different studies have already proven that the use of this method increases the sensitivity of the radiological exam significantly, especially concerning the detection of lesions of the vascular system and soft tissue. Depending on the findings, the sensitivity of the PMCT-angiography is even higher than the one of conventional autopsy. Therefore, the performance of pre-autopsy MPMCTA has already become a new gold standard, especially in cases in which the source of a hemorrhage should be detected or a modified vascular anatomy is the result of a surgical intervention.

Although the technique is well described for adult PMCT-angiography, no pediatric cases have yet been reported and no protocol has been established in order to perform this type of investigation on children. This study started to adapt this technique on infants. Two first cases of six-year-old and seven-year-old children have already been investigated by adapting values of perfusion and using the same or adapted technical equipment as for adult cases. The results of these promising experiences are reported and modifications of technical equipment and perfusion protocols based on these experiences are proposed.

This presentation shows the advantages and limitations of PMCT in investigating cases of infant death. Although it is highly advantageous for cases of traumatic death, it shows clear deficits for investigating natural death. These limitations may be overcome by performing postmortem angiography, as is already the case for adult death investigations.

Forensic Imaging, Postmortem CT, Postmortem Angiography

H134 Blast Injuries: Radiology-Pathology Correlation

Edward Mazuchowski II, MD, PhD, 115 Purple Heart Drive, Dover AFB, DE 19902; and Howard T. Harcke, Jr., MD, 3205 Coachman Road, Wilmington, DE 19803*

After attending this presentation, attendees will better understand how different radiology modalities and techniques can be used to augment the forensic pathology investigations of individuals who die in an explosive event.

This presentation will impact the forensic science community by providing information on how the results from different radiology modalities and techniques correlate to the pathologic findings at autopsy and augment the forensic pathology investigation of blast events.

Blast injuries occur when the body is subjected to an explosive event. The medicolegal death investigation of this type of event is usually complex and the injuries sustained generally vary widely from event to event and individual to individual within a specific event. The type of injuries that an individual sustains depends on many factors such as the nature of the explosive event, distance from the explosive event, and proximity of structures and moveable objects. Blast injuries are often subdivided into primary, secondary, tertiary, and quaternary blast injuries. Primary blast injuries are produced by the impact of the blast wave on the body. These injuries include rupture of the tympanic membrane, lung injury, internal organ injury, and body fragmentation. Secondary blast injuries are produced when fragments of the device or surrounding debris is accelerated by the blast wave and strikes the body. This can result in blunt force injuries, sharp forces injuries, and/or penetrating injuries. Tertiary blast injuries are produced when the body strikes an object after being accelerated by the blast wave. These injuries include lacerations, abrasions, skeletal fractures, and internal organ injury. Quaternary injuries include all of the injuries or diseases not due to the other three mechanisms, such as thermal injuries and inhalation injuries.

At the Office of the Armed Forces Medical Examiner located on Dover Air Force Base, DE, all individuals undergo full-body digital X-ray and full-body Computed Tomography (CT). In addition, fluoroscopy is available for real-time visualization of radiopaque material. Except for total body fragmentation, primary blast injuries are not readily visualized with fluoroscopy and digital X-ray. In contrast, multiplanar 2D and 3D images generated using CT can depict injury to the lungs and internal organs. Radiopaque fragments within the body due to secondary blast injuries can be depicted with fluoroscopy and digital X-ray; however, it is necessary to obtain orthogonal images in order to determine the precise location. CT readily depicts the precise location of the fragment and can give an indication of the type of material. Tertiary blast injuries such as skeletal trauma can be depicted with all three modalities. With the use of directed angiography, CT can depict vascular and some soft tissue injury. The findings of these radiographic studies help guide the forensic pathologist during the examination and allow for the complete documentation of the injuries sustained and recovery of any foreign material.

Blast Injury, Computed Tomography, Radiology

H135 Clinicopathologic Correlations in a Free-Dive Competition Fatality

M.G.F. Gilliland, MD, Brody School of Med at ECU, Pathology & Lab Medicine, Brody 7S-18, Greenville, NC 27858-4354; and Kerry Hollowell, MD, 1005 N Overlook Drive, Greenville, NC 27858*

The goal of this presentation is to introduce the first pathological examination of the effects of pressure-related injury on the lungs during competitive free-diving and the disastrous consequences of repetitive lung squeeze.

This presentation will impact the forensic science community by demonstrating for the first time the pathological changes that may occur when a competitive free-diver repetitively exposes his lungs to injury.

This is an important investigation for the free-diving community as this death is the first death of a free-diver in a competitive situation in the history of competitive free-diving. Its implications impact our understanding of the physiological and pathological changes that occur when exposing the lungs to repetitive pressure-related injury. This will impact the forensic science community by introducing the pathological findings seen in such injuries in the event that another death occurs.

Pressure-related lung injury is well known in the diving community with many factors playing a role in susceptibility to such an injury. Known risk factors include: diving too fast to depth, decreased flexibility of the chest wall, and stress. Before this pathological examination, only signs and symptoms seen in divers experiencing a lung squeeze were known. The clinical symptoms include hemoptysis and shortness of breath. The diving community has not known the extent of damage occurring at the tissue level with repetitive lung squeezes and the consequences of this tissue damage to both future diving and in individual health.

A 32-year-old male with no significant past medical history was competing in an international free-diving competition. He was the first American to ever reach 100m on one breath of air and at the time of his death was considered to be in good health. He attempted a 74m dive without fins 48 hours after a previous dive had resulted in hemoptysis immediately after the dive. He developed respiratory distress 30 seconds after surfacing under his own power. His face was not submerged as rescue divers assisted and helped him to a platform where a physician was available to assist him. Despite her efforts, he progressed to respiratory arrest and subsequent death. An initial medicolegal autopsy identified acute lung injury complicating barotrauma. Intra-alveolar hemorrhage was identified with both acute hemorrhage and hemosiderin-laden macrophages that were seen. Additional questions remained in the first-ever reported death in a free-diving competition. Better understanding of the pathologic changes occurring after repetitive injury was sought by the sponsoring agency and the diving community in order to prevent further deaths from this type of injury. The family authorized a second autopsy examination of the viscera preserved at the medicolegal autopsy.

A pathological examination was undertaken as the first direct study of pressure-related lung injury from deep breath-hold diving. Repeated barotrauma causes recurrent pulmonary hemorrhage and acute edema, hemorrhagic edema, and hemosiderin macrophages seen most extensively in the periphery of the lungs and near the conducting airways. The alveolar capillaries were found to be the source of the pulmonary hemorrhage. Thorough examination of the available airways revealed no proximal source of hemorrhage. Mild interstitial fibrosis was seen in the areas with more extensive hemorrhage. Additionally, mild fibrointimal thickening of the pulmonary arteries was identified with right ventricular hypertrophy — markers of pulmonary hypertension. The physiological changes occurring in response to repetitive barotrauma lead to pulmonary alveolar and vascular remodeling, increasing the risks in subsequent dives.

Ultimately, this diver had developed enough physiologic derangement in response to repetitive barotrauma that he had no reserve when placed under the stress of a long and deep dive. The understanding of this process will bring change to the sport of free-diving and a better understanding of the limits of the human body when placed under stress and pressure over time. Understanding the pathologic changes that occur with this type of injury will direct the care of people experiencing similar symptoms and may help them make a more educated decision whether or not to continue diving after such an injury. Knowing the pathologic changes will direct further research to better understand human physiological adaptation and the potential for serious injury.

Pulmonary Barotrauma, Free-Dive Competition, Pathology of Lung Squeeze

H136 Human Fatalities Due to Animal Attacks: A Six-Year Study From 2009 to 2014 in the Vidarbha Region of Maharashtra, India

Nilesh K. Tumram, MD, 85 Anantnagar, Nagpur, Maharashtra 440013, INDIA*

After attending this presentation, attendees will able to evaluate the rise in occurances of human fatalities caused by animals, particularly larger animals, whether wild or domesticated, such as tigers, leopards, bears, boars, dogs, and bulls.

This presentation will impact the forensic science community by raising awareness for attendees and animal activists who encourage wild population animal growth of an increase in human/animal clashes as a result of shrinking green cover and unabated encroachment of forest fringe areas.

The Vidarbha region of Maharashtra, India, is known for its forest and tiger population. Nagpur city has been rightly declared the tiger capital of India. The increase in the number of tigers, leopards, bears, wild boars, etc. in this region may be cause for animal activists to cheer, but the rise in human deaths due to incidents of man-animal conflict has been causing concerns to the authorities. Such a scenario might also be quite common in other regions worldwide.

News of people dying due to animals entering human settlements are on the increase. Recently, local people in forest areas have put pressure on officials to kill tigers and leopards that stray into their settlements. Similarly, there is a rise in the population of stray animals in rural and urban regions. Also, domestic and pet animals share a good percentage of the animal population interacting with human lives and during the handling of such animals, humans may get injured and some may even die.

This presentation will deal with the age and sex distribution of human fatalities corresponding to animal attacks. The common sites of attack and cause of death will be elaborated upon. The probable reason behind human and animal conflicts will be explored. Similarly, the injury pattern caused by different animals on human subjects will be analyzed. The medicolegal death investigator should become more familiar with the patterns of such injuries and deaths caused by these animal-human conflicts.

Human Fatality, Animal Attack, Investigation

H137 A Quantitative Assessment of Peri-Mortem Blunt Force Trauma of the Neck

Deborrah C. Pinto, PhD, Harris County Institute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054; and Deanna Oleske, MD, Harris County IFS, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will understand the variation in injury patterns observed in cases of blunt force trauma to the neck; in particular, the frequencies at which these injuries can occur in association with different manners of death.

This presentation will impact the forensic science community by contributing to the understanding of neck structure trauma and may provide empirical support for the differential diagnosis of various types of blunt force trauma to the neck.

Neck injuries, to both soft and hard tissues, are well known to be associated with blunt force trauma, particularly that resulting from strangulation, hanging, direct impacts, or indirect impacts to the neck region. There still exists a paucity of information regarding the location and frequency of these injuries in non-experiment-based research, particularly with respect to the hard tissue trauma. The goal of this study is to examine all cases of traumatized hard and soft tissue neck structures in order to identify distinctive patterns that may occur in relation to the mechanism of death. Associations between blunt force injury patterns and mechanisms are explored.

A retrospective review of autopsy reports completed at the Harris County Institute of Forensic Sciences during 2012-2014 that included an anthropology analysis of peri-mortem blunt force trauma was conducted. The location and types of soft and hard tissue injuries as well as cause and manner of death were recorded. The injury locations were grouped into three general areas: (1) external (cutaneous soft tissue injuries); (2) internal (subcutaneous and musculature soft tissue injuries); and, (3) hard tissue (bone and cartilage injuries). The distribution of cases by manner of death was as follows: 9 accident, 70 homicide, 13 natural, 12 suicide, and 10 undetermined.

Only 12 of the reviewed cases had no evidence of any soft or hard tissue injury. A small number of cases (7%) could not be assessed for soft tissue injuries due to the advanced stage of decomposition. Soft tissue injuries were observed in nearly all of the cases. In these cases, approximately 75% had external injuries and 73% had internal injuries. External injuries were present in all manners of death in varying numbers, with homicides and suicides having the highest percentages, 87% and 100%, respectively. Internal injuries were also present in all manners of death in varying numbers; however, no pattern was discerned.

Only half of the cases had hard tissue trauma with all accident cases being atraumatic. Nearly half of the total injuries were observed on the thyroid cartilage. With the thyroid cartilage fractures, most injuries occurred on the superior thyroid horn; specifically, along the base of the horn. Cricoid fractures were most frequently observed on the anterior arch. Most of the hyoid fractures were located on the greater horns; in particular, along the shaft/body.

As expected, external and internal injuries occurred in high frequencies with at least one area (i.e., external, internal, and/or hard tissue) represented. Unexpectedly, hard tissues injuries in the absence of external injuries were also present in cases that were ruled as homicide, natural, and undetermined. Similarly, hard tissue injuries in the absence of internal injuries were present in cases with all manners of death represented, except for accident. With respect to soft tissue injuries, bilateral anterior neck hemorrhages were found in 39% of the cases and bilateral cartilage hemorrhages were found in 18% of the cases; however, only 24% of the total cases had bilateral hard tissue injuries. The most frequent of these bilateral injuries was observed in the cricoid cartilage. These hard tissue bilateral fractures were found in homicide and suicide cases only.

The results of this study demonstrate the importance of examining both the soft and hard tissues of the neck in order to assess peri-mortem blunt force trauma. Soft tissue has a higher tendency to be injured; however, hard tissue trauma can be observed in the absence of soft tissue trauma. Interestingly, bilateral hard tissue injuries were found in high frequencies in homicide cases and lower frequencies in suicide cases, but in no other manners of death.

Blunt Force Trauma, Neck, Larynx

H138 Anatomical Larynx Variations and Hyoid and Thyroid Fractures

Joao E.S. Pinheiro, MD, Instituto Nac Medicina Legal e Ciências Forenses, Largo da Sé Nova, Coimbra 3000-213, PORTUGAL; Jose L. Cascallana, PhD, Instituto de Medicina Legal da Galicia, Lugo, SPAIN; Benito Lopez de Abajo, MD, Instituto de Medicina de Galicia, Santiago de Compostela, SPAIN; Xose L. Otero, PhD, Department of Statistics and Operations Research, Faculty of Medicine, Santiago de Compostela, SPAIN; and María Sol Rodríguez-Calvo, PhD, University of Santiago de Compostela, Calle san Francisco, CP 15782, Santiago de Compostela, SPAIN*

After attending this presentation, attendees will better understand anatomical variations that may influence the diagnosis of fractures of the hyoid bone and thyroid cartilages.

This presentation will impact the forensic science community by increasing awareness of the pitfalls associated with anatomical variations of the hyoid bone and the thyroid cartilage in strangulation cases, based on the prevalence of these variations established for a Galician population in northwestern Spain.

Apart from a basic anatomical background and technical skills, forensic pathologists are in general well trained in recognizing postmortem artifacts encountered during neck dissection. Unfortunately, anatomical variations as pitfalls in the interpretation of fractures of the hyoid bone and thyroid cartilage are unknown to most. This may come as no surprise, considering that forensic textbooks and forensic literature have failed to pay attention to these anatomical variations, namely with epidemiological studies assessing their prevalence.

This study was conducted on 207 consecutive autopsies looking for anatomical variations that could influence the diagnosis of larynx factures. The cartilage tritícea was confirmed as the most frequently present variation (52.7%), but different and not previously described variants were also found, such as the thyroid superior horns segmentation (11.7%), thyroid ectopic horns (8%), and thyro-hyoid lateral ossification (5.3%). Calcification of the stylohyoid ligament was the least prevalent variation found (1.4%). The names proposed for the new variants will be analyzed and discussed, with consideration given to the available literature and the probable embryologic origin from the third and fourth branchial arch.

Anatomists have described some of these anatomical variations that are of great interest to forensic pathologists. The tritícea, a very small cartilage located in the thyroid-hyoid membrane, can easily be mistaken for a fracture of the superior horns of the thyroid cartilage. The unilateral absence of one thyroid horn has also been described but not associated to ectopic horns lost in the thyro-hyoid membrane. These thyroid ectopic horns also constitute pitfalls in the interpretation of fractures of the thyroid cartilage as well as the segmentation of the distal end of the superior thyroid horns. As the thyro-hyoid lateral ossification — a thick and bony direct union between the hyoid and thyroid horns — often has discontinued/interrupted points, it can also lead to mistakes in the evaluation of thyroid fractures. The consistency of the hyoid bone and thyroid cartilage in relation to the victim's age and the degree of fusion of the corpus-horns of the hyoid should also be taken into consideration in the interpretation of autopsy findings.

Forensic pathologists should be aware of the anatomical variations of the hyoid bone and thyroid cartilage and should be trained in recognizing them in order to avoid erroneous interpretation of autopsy findings. The role of X-ray and computed tomography as ancillary techniques is increasing, but the proper manual dissection, with observation and palpation of the fractures, remains the greatest tool for forensic pathologists, whose importance will be reinforced.

Despite the tremendous importance of correct interpretation of anatomical variations in the identification of fractures of the neck structures in strangulation, this issue has not yet been properly discussed in the forensic literature, especially in terms of epidemiologic relevance. This presentation seeks to fill this gap.

Cartilage Tritícea, Thyro-Hyoid Lateral Ossificati, Thyroid Ectopic Horns

H139 Social Media and Medicolegal Death Investigation: Logged in … to the Morgue

Lorenzo Gitto, MD, Sapienza University, Dept of Anatomy, Histology, Forensic Medicine, & Orthopedics, Viale Regina Elena, 336, Rome 00169, ITALY; Stephen J. Cina, MD, 505 N Lake Shore Drive, Unit 2701, Chicago, IL 60611; Ponni Arunkumar, MD, Cook County MEO, 2121 W Harrison Street, Chicago, IL 60612; Matthew F. Fox, MD, Rush University Medical Center, 1653 W Congress Parkway, Chicago, IL 60612; and Serenella Serinelli, MD, Sapienza University, Dept of Anatomy, Histology, Forensic Medicine, & Orthopedics, Viale Regina Elena 336, Rome, Lazio 00169, ITALY*

After attending this presentation, attendees will understand the usefulness and potentiality of social networks in forensic practice.

This presentation will impact the forensic science community by demonstrating how social networks can be used to clarify certain aspects of medicolegal death investigation, with special attention paid to establishing the postmortem interval, mental/emotional state of the victim prior to death, and the circumstances leading up to a fatality.

A social network is a website that allows you to connect with friends and family, share photos, videos, music, and other personal information with either a select group of friends or a wider group of people. According to the statistical data of July 2015, the most popular social network sites are Facebook® (with 1.44 billion active users), followed by Twitter® (with 302 million active users). Social network services stimulate users to create a list of "friends," to update their "status," to upload photos, to comment on other users' statuses and contents, to indicate that they like another user's content, to send private messages, and to share the current location in their profiles. All these data are usually available to the user's contacts and friends. Sometimes, users decide to make their profile available for all other social network users, sharing with them all of their personal and demographic information.

In this study, the investigative narratives stored in the Cook County Medical Examiner's Office electronic database (LabLynx eLab solution) between August 2014 and July 2015 were searched for the following keywords: Facebook®, Twitter®, Tweet, Instagram™, IG, LinkedIn®, Snapchat™, and YouTube®. The word "Facebook®" was found in 15 reports including five suicides, four homicides, four accidents, and two natural deaths. The word "tweet" was found in one report regarding a case of suicide. No other matches were encountered. All of the reports were useful in either establishing the postmortem interval or elucidating upon the circumstances surrounding death. Specifically, they assisted the investigators in documenting intent for suicides, predicting natural deaths, detecting subjects who bragged about committing homicide, identifying instigated confrontations leading to homicide, and establishing altered mentation through bizarre postings in cases of overdose.

The incredible amount of freely available data on social media platforms can be integrated into a thorough medicolegal death investigation. Reviewed postings from decedents may assist the investigator in refining: (1) time of death, because every comment, status update, post or other activity is time stamped. Activity means the victim was alive at a given moment; (2) circumstances surrounding death, as postings may be used to reconstruct a timeline leading to death, provide evidence of intoxication, or elucidate motivation for violent acts; and, (3) manner of death, as "virtual suicide notes," complaints about ill health or symptomatology, and statements regarding fear of potential assailants may be encountered.

Social networks are a relevant tool for the death investigator, despite the possibility of online impersonation (false profiles) and potential difficulty in evaluating the actual authenticity of the messages. The forensic investigator must be aware that not all information found on profile pages is accurate but, despite these limitations, "cyberbiographical data" on social network sites should be integrated into a thorough medicolegal death investigation.

Social Media, Medicolegal, Death Investigation

H140 What is Sex? Autopsy Documentation and Death Certification in the Transgender Population

Jan M. Gorniak, DO, Office of the Chief Medical Examiner, 401 E Street, Washington, DC 20024*

After attending this presentation, attendees will be able to identify which states allow transgender individuals to amend their birth certificates. Attendees will be able to describe how a transgender person's sex should be documented in an autopsy report. Attendees will better understand what documentation is needed to complete the death certificate of a transgender person.

This presentation will impact the forensic science community by recognizing the importance of proper death certification in the transgender population. As 41% of the transgender community surveyed has attempted suicide and suicide deaths are investigated by medical examiners and coroners, it is likely that transgender individuals will be examined by forensic pathologists.

There are approximately 700,000 transgender people in the United States, or 0.3% of the adult population. Forty-one percent of transgender people surveyed in Injustice at Every Turn said they had attempted suicide, compared with 1.6% of the general population. Those who reported bullying, sexual assault, and job loss were at an increased risk.[1]

Dictionary.com defines sex as either the male or female division of a species, especially as differentiated with reference to the reproductive functions. Gender is defined as either the male or female division of a species, especially as differentiated by social and cultural roles and behaviors.[2] At autopsy, forensic pathologists usually rely on anatomical description of an individual to assign sex. Thus, if a patient identifies as a male, but a uterus and ovaries are present, the person is described as being female. Presently, the terms "transgender male" or "trans male" should be used to describe female-to-male transgender people and "transgender female" or "trans female" should be used to describe male-to-female transgender people.[2] Historically, whatever sex was described at autopsy was then entered onto the death certificate.

Amending the sex designation on a birth certificate may be an extremely important step for a transgender person to accurately reflect on this legal document the sex with which the individual identifies. A majority of states permit the name and sex of a transgender individual to be changed on a birth certificate, either through amending the existing birth certificate or by issuing a new one. Only Idaho, Kansas, Ohio, and Tennessee refuse to change the gender marker on a birth certificate.[3]

In addition to the physical characteristics noted at autopsy and initial case information, next-of-kin or family opinion may be useful in determining how a deceased person identified themselves; however, many transgender people are estranged from relatives who are uncomfortable with their gender transition. Amended birth certificate, driver's license, or passports may be sufficient legal documentation to trump family opinion. Therefore, medical examiners and coroners should record a person's gender identity rather than anatomical sex on a death certificate.

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Transgender, Amended Birth Certificate, Death Certificate

H141 The Current System of Forensic Science Inspection and Accreditation in China

Zhaoming Guo, MD, Institute of Evidence Law and Forensic Science, NO.26, Houtun South Road, Haidian District, Beijing 100192, CHINA; Ling Li, MD*, OCME, State of Maryland, 900 W Baltimore Street, Baltimore, MD 21223; Tiantong Yang*, Haidian District, 26 Houtun South Road, Beijing 100192, CHINA; and Xiang Zhang, MD*, OCME, 900 W Baltimore Street, Baltimore, MD 21223*

After attending this presentation, attendees will better understand the current system of Chinese forensic science inspection and accreditation.

This presentation will impact the forensic science community by providing attendees with a better understanding of the role and scope of forensic experts in the Chinese forensic science accreditation program. This presentation will also demonstrate the difference between China and the United States in the field of medicolegal investigation system and in their accreditation programs.

Many countries have introduced laboratory inspection accreditation programs in order to regulate forensic scientific services and improve the quality of forensic/medicolegal investigation. In 2005, China's National People's Congress Standing Committee adopted "The Decision on Forensic Science Management Issues." The forensic scientific laboratory accreditation program was then formally established as a quality assurance agency in China.[1]

China National Accreditation Service for Conformity Assessment (CNAS) is the sole agency authorized to inspect and accredit forensic institutions with international accreditation standards. The quality and technical administrative departments act as implementing agencies of the traditional accreditation activities at province level, which is under the management of the Certification and Accreditation Administration of the People's Republic of China (CNCA).

In 2013, the CNAS released the *CNAS-CL08: 2013 Accreditation Criteria for the Competence of Forensic Institutions*, which is based on the International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 17025 criteria and ISO/IEC 17020 criteria, combined with the characteristics of the Chinese forensic investigation system.[2,3] The CNAS also released seven related accreditation standards in April 2014.

In the past decade, the number of accredited forensic scientific institutions increased significantly. There were only five institutions accredited by the international standard 12 years ago. Increasing by 47 times, the number of accredited forensic institutions in 2013 was 237.

The CNAS has 15 subcommittees. Currently, the CNAS carries out inspections and accreditation in four major forensic areas, including: (1) forensic medicine including forensic pathology, forensic toxicology, clinical forensic medicine, and forensic psychiatry; (2) evidence analysis including forensic serology/DNA analysis; (3) audio-image analysis; and, (4) electronic data analysis.

The CNAS is a peer-review system. The inspectors are forensic experts in their respective fields. There are 350 inspectors nationwide. The Chinese forensic science proficiency testing program has been used in all areas of forensic science accreditation. The Ministry of Public Security and the Supreme People's Procurator implement verification under the authority of CNAS every year.

This study presents the current status of the Chinese forensic science inspection and accreditation system. The accreditation of forensic science is intended to evolve over time. It is believed that the Chinese forensic science inspection and accreditation system will develop into a new era.

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Forensic Science Inspection, Accreditation, Chinese Systems

I1 Cyberbullying: The Violence Behind Technology and Implications for Adolescents' Self-Esteem

Ana Rato, MS, Faculty of Medicine, University of Porto, University of Porto, Porto, PORTUGAL; Celina Manita, PhD, Faculty of Psychology and Education Sciences, University of Porto, Porto, PORTUGAL; Ricardo Jorge Dinis-Oliveira, Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, Porto 4200-319, PORTUGAL; and Teresa Magalhães, PhD, Faculty of Medicine, University of Porto, Porto, PORTUGAL*

After attending this presentation, attendees will better understand some of the relevant outcomes of cyberbullying for adolescents, which are necessary to know when attempting to achieve timely detection, reporting, and reducing or avoiding negative outcomes.

This presentation will impact the forensic science community by illustrating how cyberbullying is a threat adolescents may experience and how this may have a profound influence on behavior, personality, and self-esteem, and can even culminate in suicide.

Today's adolescents are part of the first generation who have grown up in societies in which the internet is an integral part of their lives and is related with many daily activities.[1-4] With the development of these new forms of communication, adolescents expanded their social network beyond the physical world, interacting and making friends with people they do not personally know.[2] These aspects increase the risk of a new type of bullying — cyberbullying, which is often referred to as one of the main threats adolescents have to incur. Therefore, the effects of this phenomenon on adolescent behavior has become an important field of investigation; however, there is still little knowledge about the consequences and many inconsistences are found in the literature regarding cyberbullying.[5]

This exploratory study examines the association between the experience of cyberbullying and the perception of self-esteem, as well as if the way to cope with this victimization is associated with a lower perception of self-esteem. To collect this data, 20 institutions (schools and study centers) of the second-largest Portuguese city (Porto) were contacted and 466 Portuguese adolescents between 15 and 20 years of age were enrolled in the study. The instruments used in this study were the Rosenberg self-esteem scale, to evaluate self-esteem, and a survey about cyberbullying. Results showed a statistically significant association between cyberbullying victimization and low self-esteem. Moreover, results proved that cyber-victims who cope with cyberbullying by hiding the problem and choosing to do nothing present with a lower perception of self-esteem than those who cope in other ways. Schools, educators, parents, and adolescents need to be aware of this form of violence and its consequences. Therefore, it is important to continue to investigate and discuss the creation of prevention and intervention programs that can address this phenomenon.

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Cyberbullying, Adolescents, Self-Esteem

I2 Fatherhood and Incarceration: Primary Results on Parenthood and Imprisonment

Susanna Pietralunga, PhD, University of Modena e Reggio Emilia, Via Università, 4, Modena 41100, ITALY; Alessandro Taurino, PsyD, University of Bari, Piazza Umberto I, Bari 70124, ITALY; Rosalinda Cassibba, PsyD, University of Bari, Piazza Giulio Cesare, Bari 70124, ITALY; Giuliana Lacalandra, PsyD, University of Bari, Piazza Umberto I, Bari 70124, ITALY; Elisabetta Preti, PsyD, University of Modena e Reggio Emilia, Via Università, 4, Modena 41100, ITALY; Maria Pasceri, PhD, University of Modena e Reggio Emilia, Via Università, 4, Modena 41100, ITALY; Gianmichele Laquale, PhD, University of Bari, Piazza Umberto I, Bari 70124, ITALY; Alessio Ostuni, MD, Sections of Legal Medicine and Criminology, Policlinico of Bari Italy, Piazza Giulio Cesare 11, Bari 70124, ITALY; Nicola Petruzzelli, PhD, Corso De Gasperi 306, Bari 70124, ITALY; Anna Cassano, PsyD, University of Bari, Piazza Giulio Cesare, Bari 70124, ITALY; Roberto Catanesi, MD, p.za G. Cesare, Bari 70124, ITALY; and Ignazio Grattagliano, PsyD, University of Bari, Piazza Cagnola, 3/B, Casamassima, Bari 70010, ITALY*

After attending this presentation, attendees will better understand and appreciate the importance of the impact of incarceration on the father-child relationship and the need to create programs to address this dilemma.

This presentation will impact the forensic science community by increasing awareness of the importance of both the incarcerated father and his child/children when creating a rehabilitation plan.

When a father is incarcerated, his role as parent becomes "at risk" as being in detention undermines some of the fundamental aspects associated with being a parent. Going to prison alters the reciprocal nature of parent-child interactions. A father in prison cannot fully carry out his role as parent because, under such conditions, he is not able to impart a sense of attachment, trust, and security that is fundamental to the child's development. In addition, stereotypes and prejudices may contribute to painting a picture of the incarcerated father as one who is unable to be a good parent. This could result in a life of failure and feelings of inadequacy with regard to being a father and parent. Furthermore, the absence of adequate role models, the very difficult initial adjustment period to prison life, the lack of cognitive, communicative, and relational abilities, together with the restrictive context of the prison, all make it difficult to develop and maintain adequate father-child ties that are so vital to a child's development. Simply put, prison conditions alter both the parent-child relationship and how the subject perceives himself as a father and parent.[1-3]

Thus, there can be no doubt as to the importance of corrective interventions that address such negative dynamics and to the importance of support initiatives for prisoners and their families where specified locations and times for meetings between father and child can take place. These environments must be appropriate for developing and maintaining relational continuity, as well as for establishing and promoting a sense of parental responsibility in the incarcerated parent.[4-6]

The objectives of the study are to: (1) establish the father's perception of his role as a parent, (2) establish the attachment styles of incarcerated fathers; and, (3) explore the relationship between the self-perceived parental role and the attachment patterns of the study subjects.

Method: The directors of penitentiary administrations from two Italian regions were involved in this study. One hundred fifty male inmates were enrolled, each of whom was asked to give informed consent. Every participant was administered an articulated medical history questionnaire, in addition to two parental competence evaluation instruments.

Instruments: Attachment Style Questionnaire (ASQ) and Self-Perception of Parental Role Questionnaire (SPPR).[7]

Final Considerations: The capacity of minors to establish multiple, deep attachments with people, even those who are not part of the immediate family circle, is well known. This is especially true when such figures demonstrate availability and readiness to respond to a child's signals. As a result, a child's social network takes on great importance as the child develops, particularly for children whose parents are in prison. This is linked to the correlation between successful prison re-education strategies and the ability to maintain a good relationship between the detained, his children, and his family.

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Incarcerated Parents, Prison Rehabilitation, Parental Attachment

I3 Traumatic Exposure and Competency to Stand Trial: Describing Juvenile Offender Characteristics

Sheresa Christopher, PhD, Medical University of South Carolina, 29 Leinbach Drive, Charleston, SC 29407; Christopher Fields, MD*, Medical University of South Carolina, 29 C Leinbach Drive, Charleston, SC 29407; Diana Mullis, MD*, Medical University of South Carolina, 29-C Leinbach Drive, Charleston, SC 29407; and Jennifer Steadham, PhD, Medical University of South Carolina, 29-C Leinbach Drive, Charleston, SC 29407*

After attending this presentation, attendees will understand characteristics typically associated with juvenile offenders referred for competency to stand trial evaluation and will have better knowledge of the relationship between trauma and offending behavior in such youths.

This presentation will impact the forensic science community by bolstering understanding about the characteristics of legally involved youths. Forensic examiners and legal professionals alike will benefit from increased understanding of the populations they serve and the nature of their offending behavior.

Exposure to traumatic events has been found to be associated with deficits in emotion regulation, social information processing, and increased anger and aggression.[1-3] These same characteristics are also found within populations of delinquent youths.4 Within the juvenile offender population, a small subset of youths is referred for evaluation of their competency to stand trial due to concerns they may lack a factual and rational understanding of the proceedings against them and the ability to assist their attorney in their defense. Many researchers have found that childhood victimization may be a risk factor for engaging in delinquent behaviors.[2] Additionally, rates of trauma are drastically higher in populations of legally involved youths than in the general population. For example, while 5% of a large sample of adolescents met criteria for post-traumatic stress disorder, rates have been found to range from 24% in a sample of detained youths to 49% in a sample of delinquent adolescent females.[5-7] Lastly, exposure to trauma has been found to be associated with deficits in development and cognition, emotional experience, and behavior.[1,3,8] A well-known trauma expert wrote from a theoretical perspective and describes how repeated trauma has "pervasive effects on the development of the mind and brain."[1] The aforementioned deficits are often cited as the basis for seeking evaluation of a juvenile's competency to stand trial.

Despite the high prevalence of trauma exposure and the similarity of deficits observed, little is known about trauma exposure in youths thought to exhibit deficits in those abilities typically associated with competency to stand trial. The current study seeks to describe the differences in characteristics between juveniles who are opined competent to stand trial and those who are not. A particular emphasis is placed on the presence and type of past trauma exposure in relation to the nature of the criminal offenses given the high prevalence of trauma in this population. A sample (*N*=25) of juvenile competency to stand trial evaluations completed in the past three years was examined. Data was coded with regard to type of past trauma exposure, offense type, and several other variables pertinent to the question of interest. Furthermore, data regarding the deficits identified and overall opinion with regard to competency to stand trial is described. Data extracted from these documents were analyzed via descriptive statistics and correlation research methods will be used to describe the nature of the relationship between trauma and offending behavior in this subset of youths. Strengths, limitations, and implications of data obtained will be discussed.

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Juvenile, Competency, Trauma

I4 Women Accused of Sexual Abuse: Three Case Reports From Turkey

Esra Unal, MD, Adli Tip Kurumu, Istanbul, Bahcelievler, TURKEY; Volkan Unal, MD, Adli Tip Kurumu, Istanbul, Sirinevler, TURKEY; Tuba Özcanli, Adli Tip Kurumu Çobançesme M. Kimiz S. No:1, Bahçelievler/Yenibosna, Istanbul, TURKEY; Murat Imali, Council Of Forensic Medicine Bahçelievler, Istanbul 34196, TURKEY; and Ibrahim Balcioglu, Istanbul Niversitesi, Cerahpasa, Tip Fakultesi, Psikiyatri Anabilim Dali, Istanbul, TURKEY*

WITHDRAWN

I5 Made Up by Makeup — Pretense of an Offense

Sabrina Mauf, Institute of Forensic Medicine, Winterthurerstrasse 190/52, Zuerich 8057, SWITZERLAND; Rosa M. Martinez, MD, Institute of Forensic Medicine, Winterthurerstr 19052, Zurich 8057, SWITZERLAND; and Christine Bartsch, MD, Winterthurerstr 190/52, Zurich 8907, SWITZERLAND*

After attending this presentation, attendees will be aware, with the assistance of clinical pictures, of the existence of a special case of victim role staging. Claiming a criminal offense by using painted-on injuries is an exceptional case and is rarely seen. Attendees will be sensitive to such scenarios and may integrate this knowledge into their daily routine.

This presentation will impact the forensic science community by creating an awareness of criminal offense-staging using artful make-up skills. This case highlights the psychiatric aspects in forensic medicine and demonstrates the importance of an interdisciplinary approach.

Introduction: Self-inflicted injuries are a common topic in the field of forensic medicine. In particular, the differentiation of these injuries from those incurred by a third party is crucial; however, self-painted injuries created with makeup, which entail misleading the medical staff and the administration of justice, seems to be a rarity. To date, no such case can be found in the literature.[1]

Case: A 26-year-old female filed a complaint against unknown individuals after she had supposedly been robbed a few hours previously. She reported that two unknown men unexpectedly strangled her as well as punched and kicked her in her face and body before stealing money from her. Later, the forensic clinical examination was ordered by the police and prosecutor.

The woman showed subjective symptoms, such as tenderness of the head and torso, and objective symptoms, such as numerous diffuse, red-violet skin discolorations of the face, neck, torso, and extremities. The discolorations had the appearance of bruises. Yet, because of a noticeably pasty skin appearance resembling normal makeup application, the forensic expert removed the makeup from the pertinent regions of the body. As a surprising result, all discolorations could be eliminated and, therefore, the "injuries" were discovered to be made up. Actual recent or fresh injuries, such as bruises, could not be found. Thus, indications for a third-party interference did not exist. According to the findings of the psychiatrist, emotionally unstable personality traits may be possible factors for this victim's role staging. Furthermore, police investigations disclosed frequent reports against unknown persons filed by the same woman in the past. Toxicological analyses did not reveal any foreign substances in her blood or urine samples.

Conclusions: The reported case represents a rare type of victim role staging and misleading of medical staff and the administration of justice. There are some similarities as well as certain differences to the previously described aspects of self-inflicted injuries.[1,2] From a forensic and psychiatric perspective, parallels to the Munchhausen syndrome can be drawn and indications of emotionally unstable personality traits can be found.[3] The present case creates awareness of the option of this type of staging and also demonstrates the need for an interdisciplinary approach.

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Self-Inflicted Injuries, Victim Role Staging, Forensic Psychiatry

I6 Ethical Responsibilities of Physicians: Capital Punishment in the 21st Century

Robert Weinstock, MD, 1823 Sawtelle Boulevard, Los Angeles, CA 90025; William C. Darby, MD*, UCLA, 760 Westwood Plaza, C8-193, Los Angeles, CA 90024; Chinmoy Gulrajani, MD*, 2450 Riverside Avenue, F-222, Minneapolis, MN 55454; and Karen B. Rosenbaum, MD*, 49 W 24th Street, Ste 908, New York, NY 10010*

After attending this presentation, attendees will have a concept of the history of the death penalty in the United States, the practice of physician involvement in death penalty cases, and the American Medical Association (AMA) and this presentation's ethical point of view on direct participation of physicians in the death penalty.

This presentation will impact the forensic science community by illustrating the current discrepancy between the law and medical ethics with regard to capital punishment in the United States.

Per Amnesty International the United States is in the company of 22 other countries, such as Afghanistan, Algeria, and South Korea, having reported death sentences in 2014. The AMA is one of many medical professional organizations that prohibit the participation of physicians in the actual act of execution per AMA Code of Medical Ethics, Opinion 2.06. This code specifically states, "An individual's opinion on capital punishment is the personal moral decision of the individual. A physician, as a member of a profession dedicated to preserving life when there is hope of doing so, should not be a participant in a legally authorized execution." Despite these clear guidelines for physicians, there is debate within the medical field about physician involvement in various aspects of death penalty cases.

As recently as June 29, 2015, in *Glossip vs. Gross*, the United States Supreme Court upheld the death penalty, specifically the use of the controversial sedative midazolam. The injection of this sedative had contributed to several botched executions. For lethal injection to be considered humane, there must be physician involvement. Historically, there has been physician involvement in executions. The three-drug regimen used in lethal injections was initially proposed by a physician. Forensic psychiatrists have a primary duty to answer legal questions presented to them to foster justice; however, they also have a secondary duty as physicians to do no harm. In death penalty cases, physicians have been asked to participate as expert witnesses by both the defense and the prosecution in various capacities. In this presentation, different positions that physicians and specifically forensic psychiatrists have taken on this issue will be outlined. This presentation's position is that given the overwhelming secondary duty related to their physician role and biomedical ethical considerations, specifically to do no harm, forensic psychiatrists should not utilize their expertise if they believe their involvement will be used for the primary purpose of obtaining a death penalty.

In this presentation, there will be an overview of the history of the death penalty, a discussion of the use of lethal injection and physician involvement, forensic psychiatrist's participation in death penalty cases, the problem of execution of mentally ill individuals, and the position this study takes on participation of physicians in death penalty cases. There will also be a discussion regarding the above ethical issues.

Capital Punishment, Ethics, Death Penalty

I7 The Forensic Quality Challenges of the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5TM) and Neuroscience

John L. Young, MD, 203 Maple Street, New Haven, CT 06511-4048*

After attending this presentation, attendees will be better able to utilize the *DSM-5TM* to embrace the challenges of evaluating biological advances in neuroscience on several fronts.

This presentation will impact the forensic science community by offering a basis for keeping performance up-to-date and improving as advances take place in psychiatric nosology and behavioral science.

The mental health profession expected the *DSM-III* to provide an opportunity for the enhancement of psychiatric research. Remaining neutral with regard to causality, it provided a disciplined phenomenological approach for reliably reducing symptom-based criteria to distinct diagnoses. Researchers and clinicians alike expected to see significant advances in the understanding and treatment of mental illnesses. This would become possible since all professionals, including forensic behavioral scientists, would henceforth be speaking the same language with regard to diagnosis, including both major headings and subtypes. With the study of more rigorously defined disorders that would thus become possible, it seemed reasonable to anticipate increasingly valid conclusions about their biology, their psychology, and their treatment. Not surprisingly, the *DSM-III* duly became a fixture of forensic behavioral science as well.

Some three decades later, the publication of *DSM-5TM* arrived to a rather more subdued reception, with clinicians widely predicting that they and their colleagues will largely ignore it as being little different from the *DSM-IV-TRTM*. The head of the National Institutes of Mental Health predicted a similar response from the research community, explaining that as strong as the phenomenology-based consensus may have become, it remains but a summary of opinion offering disappointingly little biology.

The urging for biomarkers to associate definitively with mental disorders has remained frustrated despite many advances in research on brain and mind. Experts have explained this disparity in terms of both the powerful influences of culture on human behavior and the complexity of the brain itself. In particular, there was a widespread expectation that the publication of *DSM-5TM*, if not *DSM-IV*, would include diagnostic biomarkers in one or more of the dementias, an expectation that went unfulfilled.

Since then, the pace of research into mental disorders has been increasing. In addition to the dementias, especially promising areas now include the epigenetic tracing of substance abuse disorders, computer-assisted comparisons of literally thousands of brain scans, and the prospect of continuous Electroencephalograph (EEG) monitoring with minimally obtrusive headsets.

From these and other advances, some not yet predictable, forensic behavioral experts can expect challenges and opportunities. At the same time, a current set of practice guidelines from the American Academy of Psychiatry and the Law, states that withdrawing support from the *DSM* is not an option, at least in North America.[1] This is based on its widespread use and its familiarity to the legal profession. Through the responsible use of evidence, we, as a profession, can make the *DSM-5TM* a platform for contributing to progress in the understanding of the mind, normal and abnormal. We possess, through tradition and training, the potential to understand developments in the neurosciences. Thus, we can contribute to the ongoing *DSM* process as we ensure continuing quality improvement in our own work.

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DSM-5TM, **Neuroscience, Quality**

I8 Evaluating Access to Substance Abuse Treatment in a Public Hospital Setting for Persons on Probation Under California's Realignment Program

Eric Chaghouri, LAC+USC Medical Center, Department of Pyschiatry, 2010 Zonal Ave, OPD, 1P-2, Los Angeles, CA 90033; Kimberly Brown, MD*, 2010 Zonal Avenue, Los Angeles, CA 90033; Kate Taylor, PhD, LAC+USC Medical Center, Dept of Psychiatry, OPD Bldg 1P-10, 2010 Zonal Avenue, Los Angeles, CA 90033; and Kellie Spector, BS, Keck School of Medicine of USC, 1975 Zonal Avenue, Los Angeles, CA 90033*

After attending this presentation, attendees will understand how a challenging population that often has co-morbid substance abuse and mental health problems can gain access to resources for substance abuse. The demographics of the sample will be described and the various types of interventions recommended for them, as well as the barriers that affect engagement with treatment, will be discussed.

This presentation will impact the forensic science community by examining several factors that may play a role as to whether the treatment needs of persons on probation (California Assembly Bill (AB) 109) as opposed to those not on probation can be adequately addressed. This study is the first of its kind undertaken at a large urban hospital that has experienced an unprecedented influx of patients transitioning from correctional institutions to the community.

Patients with substance use disorders are an often overlooked and underfunded population in the healthcare industry today. Persons on probation may experience even more obstacles in obtaining help for their substance use disorders. California's Realignment Program will be described as will the impact it has had on public and community treatment. This presentation will examine whether persons under AB 109 who have substance use disorders are provided appropriate referrals and placement, as well as the barriers for them receiving these services.

The records (*N*=934) of a certified substance abuse counselor who contacted patients at two large county medical centers during a nine-month period (July 2014-April 2015) were evaluated. These patients were identified as having potential substance abuse problems and were referred for assessment using the Screening, Brief Intervention and Referral to Treatment (SBIRT). Among those who agreed to participate, descriptive measures including age, gender, ethnicity, substances used, co-occurring mental health diagnosis, treatment recommendation, and successful intervention were evaluated. An attempt will be made to identify patients who were under AB 109 and compare their descriptive measures to the rest of the sample.

It is believed that barriers to obtaining adequate substance abuse treatment exist in general. It is anticipated that patients under AB 109 will have more barriers to placement than those patients who are not on probation. If so, practices will need to be modified and alternative approaches to more effectively treat this marginalized population for substance use disorders will need to be evaluated.

Assembly Bill 109, Substance Use Disorder, Treatment Barriers

I9 Predicting Success: A Study of Demographic Indicators of Success in Prison Career and Technology Education Training Programs

Ronald R. Thrasher, PhD, PO Box 2662, Stillwater, OK 74076; and Kimberly Litterell, BS*, 901 S Greenwood, Yale, OK 74085*

After attending this presentation, attendees will recognize demographic and correctional factors that serve as a predictor of success in a Career and Technology Education training program.

This presentation will impact the forensic science community by providing results from the data analysis in an area with little previous research. A recent study conducted by the United States Bureau of Justice confirmed that correctional education is effective but calls for further research that will identify who benefits from these programs so more will be gained from taxpayer dollars.

This study's purpose was to identify students' demographic characteristics and correctional factors that indicate success in a Career and Technology Trades training program while incarcerated. In recent studies, it has been found that interventions that include career and technical training reduce the risk of post-release recidivism by 13% and effectively saves five dollars for every one dollar spent on correctional education.[1] Prior studies have concentrated on factors that affect the success of the correctional education program itself, including aspects of instructional organization and delivery and other types of curriculum-focused support.[2]

This study focused on the students' personal demographic characteristics that implicitly predicted an individual's probability to comprehensively benefit from the training program. The first phase of the project was performed by using a grounded-theory approach to analyze Oklahoma's Skills Centers' performance data from Fiscal Year (FY) 2007 to FY2015 within seven Oklahoma Department of Corrections facilities. The second phase of the study was performed by conducting descriptive and paradigmatic interviews of program instructors that highlighted tacit attributes of students. The final phase of this study was executed through the analysis of a cross-sectional survey that was completed by integral members of the Skills Centers system. By identifying demographic indicators of success for students in the programs, a selective enrollment process can be incorporated that will allow the system to invest resources first in those with a lower probability of recidivating, therefore increasing the state's return on investment.

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Recidivism, Career/Technology Education, Correctional Education

I10 Risk Factors and Legal Implications of Psychiatric Patient Elopement From Hospital Settings

George Elias, USC Institute of Psychiatry and Law, PO Box 86125, Los Angeles, CA 90086-0125*

After attending this presentation, attendees will better understand the risk factors associated with elopement from hospitals by psychiatric patients, the theoretical environmental risk factors for elopement on medical wards, and the legal ramifications of elopement.

This presentation will impact the forensic science community by discussing a topic that is not very well understood or studied. This presentation will highlight the need for extending the span of risk assessments to include the risk of elopement from hospital settings and, in particular, less secure wards.

Patient elopement from medical and psychiatric wards is an unfavorable event with a potential for bad outcomes including harm to the patient and/or others. There are studies that have associated adverse consequences including loss of treatment, violence, and suicide with elopement from psychiatric wards; however, the literature on elopement is generally scarce, with most papers hailing from countries outside of the United States, including Australia and the United Kingdom, with none addressing elopement from medical wards. Moreover, most of the literature is found in nursing journals with no articles approaching the topic from a forensic psychiatry perspective.

Elopement is generally viewed as a heterogeneous event associated with various risk factors. Prospective and retrospective studies have been conducted to try and determine these risk factors associated with elopement. The risk factors that have been most associated with elopement include: psychosis; previous history of elopement; young age; and medication non-compliance; however, different methodologies and definitions of elopement in the literature make it difficult to determine the positive predictive value of these various risk factors.

Patients with severe psychiatric illnesses often find themselves on medical wards due to co-morbid medical problems that require immediate attention; however, most medicine floors are generally viewed as not being as secure as psychiatric wards because there are no locked doors. Moreover, non-psychiatric physicians and nursing staff are not as accustomed to dealing with acute behavioral problems secondary to psychiatric illnesses nor to the various legal issues that surround treating unwilling patients. It is postulated that a risk assessment for elopement that takes into account the acuity of both the patient's medical and psychiatric conditions in order to determine appropriate placement should be made in the emergency department.

The purpose of this presentation is to review the associated risk factors of elopement from psychiatric wards based on the literature. Moreover, an attempt will be made to look at the differences between psychiatric and medical wards and how these differences may contribute to easier elopement from medical wards. Finally, a review of the legal literature will be presented to explore the legal ramifications of patient elopement from a psychiatric-legal perspective.

Elopement, Medicolegal, Risk Factors

I11 Stopping the Revolving Door: Identifying Factors Associated With Repeated Trial Competency Evaluations

Bipin Subedi, MD, Bellevue Hospital, 462 First Avenue, New York, NY 10016; Martin Nau, MD, 634 Leonard Street, Apt 2, Brooklyn, NY 11222; and Elizabeth P. Moreira, MA, Bellevue Hospital Center, 462 First Avenue, Room 19N45, New York, NY 10013*

After attending this presentation, attendees will understand clinical and legal characteristics of defendants who have been found unfit to stand trial two or more times on a single charge.

This presentation will impact the forensic science community by providing information on an understudied subset of incompetent defendants. This information could help direct future clinical and legal interventions in order to reduce the likelihood of prolonged detention and delayed adjudication.

The Supreme Court's 1960 decision in *Dusky v. United States* created a federal standard for competence to stand trial, establishing that a defendant must have "sufficient present ability to consult with his lawyer" and a "rational as well as factual understanding of the proceedings against him." In New York, if a defendant's competence is called into question, Article 730 of the Criminal Procedure Law (CPL 730) ensures that he or she undergoes a competency assessment before the trial process can continue. If a defendant has a felony charge and is deemed incompetent to stand trial, he or she is referred to a New York State Office of Mental Health facility for restoration of competency.

However, there is a constitutional limit to how long a defendant can be hospitalized for the purpose of restoration. In 1972, the Supreme Court ruled in *Jackson v. Indiana,* that a defendant deemed incompetent to stand trial "cannot be held more than the reasonable period of time to determine whether there is a substantial probability that he will attain that capacity in the foreseeable future." Estimating the probability that a defendant can be restored to competency has proven to be a difficult task. Past research has investigated the characteristics of restorable versus non-restorable defendants, with the goal of more accurately predicting which defendants are more or less likely to be restored. Others have examined attributes of a subset of incompetent defendants who require long-term (greater than six months) restoration as well as defendants requiring multiple competency examinations for different charges. Common factors associated with lower likelihood of competence restoration include older age, a lower level of charges, a history of a chronic psychotic disorder, and a history of cognitive impairment.

There has not been significant research into the characteristics of defendants who have been found competent but then lose competency before their case can be adjudicated. These inmate/patients can have several competency evaluations and subsequent restoration hospitalizations. This population of defendants is of particular interest because of the burden that multiple and long-term episodes of incompetence pose on the correctional and mental health systems, in addition to the civil rights concern over prolonged detention and delayed adjudication. This situation has multiple clinical, ethical, legal, and cost implications.

The purpose of this study is to identify characteristics of defendants who have been found unfit to stand trial on two or more occasions for the same charge and have had a history of hospitalization in the Bellevue Hospital Forensic Psychiatry Service. The more that is known about this subset of defendants, the more capable clinicians will be in providing targeted interventions that could reduce the length of time to adjudication.

Competency Evaluation, Fitness Restoration, Incompetent Defendants

I12 The Detection of Feigned Legal Knowledge Deficits in Defendants Undergoing Competency to Stand Trial Evaluations: The Use of the Inventory of Legal Knowledge (ILK)

Emily D. Gottfried, PhD, Charleston, SC 29403; Joyce L. Carbonell, PhD, Florida State University, 1107 W Call Street, Tallahassee, FL 32304; and B. Lee Hudson, PhD, Tallahassee, FL 32301*

After attending this presentation, attendees will better understand a specific type of malingering, feigning factual legal knowledge deficits, that defendants may employ during competency to stand trial evaluations. Attendees will learn about a commonly used measure to assess for this type of malingering, the Inventory of ILK. Finally, attendees will learn of the current state of the research concerning the ILK.

This presentation will impact the forensic science community by shedding light on a relatively common type of malingering that defendants employ during competency to stand trial evaluations. This type of malingering could go unnoticed during forensic evaluations and has been largely ignored in the forensic literature.

At an estimated prevalence of 60,000 evaluations per year, competency to stand trial evaluations are the most common type of forensic evaluations.[1] As malingering rates during these evaluations have been shown to range from 20% to 30%, the accurate assessment of malingering is extremely important.[2,3] Although many defendants feign psychiatric symptoms (i.e., hearing voices), another type of malingering which has been largely ignored in the forensic literature is feigning factual legal knowledge deficits. This is extremely problematic, as the Dusky v. United States ruling clearly specifies that a defendant must have both a factual and a rational understanding of the charges against him/her.[4]

The Inventory of Legal Knowledge (ILK) is a measure to assess for response style (i.e., feigning) during a competency to stand trial evaluation.[5] This presentation focuses on the state of the current research regarding the ILK, including a summary of research by others and a thorough discussion of the research on the ILK by this study.[6,7] For example, using samples of forensic psychiatric patients adjudicated incompetent to stand trial (*N*=130) and student simulation samples (*N*=195), data on improving the recommended ILK cut score and the use of the ILK with defendants with intellectual disabilities are presented.[8,9]

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Malingering, Inventory of Legal Knowledge, Competency to Stand Trial

I13 Occurrence of a Suicide Attempt by Penis Auto-Amputation by a Murder Suspect: A Case Report

Esra Unal, MD, Adli Tip Kurumu, Istanbul, Bahcelievler, TURKEY; Volkan Unal, MD, Adli Tip Kurumu, Istanbul, Sirinevler, TURKEY; Tuba Özcanli, Adli Tip Kurumu Çobançesme M. Kimiz S. No:1, Bahçelievler/Yenibosna, Istanbul, TURKEY; Murat Imali, Council Of Forensic Medicine Bahçelievler, Istanbul 34196, TURKEY; and Ibrahim Balcioglu, Istanbul Niversitesi, Cerahpasa, Tip Fakultesi, Psikiyatri Anabilim Dali, Istanbul, TURKEY*

WITHDRAWN

*Presenting Author

I14 Elder Abuse and Violence: Descriptions of the Phenomenon by Health Care Workers From Two Italian Hospitals

Graziamaria Corbi, PhD, via Giovanni Paolo II-Loc Tappino, Campobasso 86100, ITALY; Ignazio Grattagliano, PsyD, University of Bari, Piazza Cagnola, 3/B, Casamassima, Bari 70010, ITALY; Lidia Scarabaggio, RN, University of Campobasso, Via F. De Sanctis 1, Campobasso 86100, ITALY; Carlo Sabbà, MD, University of Bari, Piazza Giulio Cesare, Bari 70124, ITALY; Giorgio Fiore, MD, University of Bari, Piazza Giulio Cesare, Bari 70124, ITALY; Nicola Ferrara, MD, via Giovanni Paolo II-Loc Tappino, Campobasso 86100, ITALY; Roberto Catanesi, MD, p.za G. Cesare, Bari 70124, ITALY; and Carlo P. Campobasso, MD, PhD, University of Molise, Dept Medicine & Health Science, via De Sanctis, snc, Campobasso 86100, ITALY*

After attending this presentation, attendees will more fully appreciate the importance of knowing how to recognize the various signs of elder abuse and the need to take the necessary steps both in prevention and in response.

This presentation will impact the forensic science community by demonstrating that elder abuse comes in many forms, some obvious and others not so obvious. New ways to address this phenomenon must be formulated and put into practice.

Background: Elder abuse is a widespread but underestimated problem. The full extent of this difficult situation is not known due to a lack of reports and/or complaints, as well as the difficulty in identifying the early warning signs of abuse. Many forms of elder abuse exist and are psychological, economic, sexual, physical, social, and institutional in nature; however, abuse also includes neglect and abandonment. It is clear that maltreatment may arise not only through active behavior, but also through omissive behavior such as silence, underestimation, and failure to report. Knowing how to identify the characteristic signs of elder abuse is the duty of every healthcare worker and is crucial in the adoption of suitable defense measures to protect the victim as well as in dealing with the offender.[1,2]

Objective: To establish the level of awareness of this issue by healthcare workers and to understand if they are able to promptly identify the early signs of abuse and take the necessary actions to report them.

Materials and Methods: From April 1 - 30, 2015, all employees (i.e., physicians, specializing physicians in training, nurses, office support staff, social-healthcare workers, and orderlies) from the Internal Medicine Operating Unit and the Geriatrics Department at Cardelli Hospital in Campobasso (Molise) and from the Policlinico of the University of Bari "Aldo Moro" (Puglia) answered a questionnaire that was formulated by utilizing the provisions of other duly used and validated questionnaires from other international situations that are used to explore: (1) employees' awareness of the phenomenon; (2) employees' ability to recognize possible signs of abuse; (3) the prevalence of the phenomenon; and, (4) employees' awareness regarding the proper actions to take when they encounter a case of abuse.

Results: Data collection resulted in a total of 98 questionnaires administered to 142 respondents (69.0%). The majority of questionnaires were completed by females (75.5%) between the ages of 41 and 50 years of age (26.7%) and by qualified nurses (46.9%). Table 1 describes the preliminary data obtained and is broken down by unit and title of those who filled out the questionnaire. Table 2 shows distribution by sex and the age range of compilers according to the operating unit to which they belong.

Table 1

	BARI (Puglia)				CAMPOBASSO (Molise)			
	Internal Medicine		Geriatrics		Internal Medicine		Geriatrics	
Title	*Enrolled*	*Collected*	*Enrolled*	*Collected*	*Enrolled*	*Collected*	*Enrolled*	*Collected*
Physician	7	4	7	1	9	4	4	3
Physician in training	15	11	15	14	0	0	0	0
Nurses	12	11	12	10	20	16	15	9
OSS	4	3	4	1	3	1	3	1
Orderlies	2	2	2	2	2	1	2	1
Aides	2	1	2	2	0	0	0	0
Total	**42**	**32**	**42**	**30**	**34**	**22**	**24**	**14**

Table 2

	BARI (Puglia)		CAMPOBASSO (Molise)		Total
	Internal Medicine	Geriatrics	Internal Medicine	Geriatrics	
Sex (M/F)	**10/22**	**6/24**	**5/17**	**3/11**	**98**
Age					
21-30	10	10	4	0	**24**
31-40	8	5	6	2	**21**
41-50	7	8	4	8	**27**
>50	7	4	8	4	**23**
No response	0	3	0	0	**3**

*Presenting Author

Conclusions: These preliminary data show that interest in elder abuse, even when present, is neither a priority for all healthcare workers nor is it perceived as a problem by them. This is probably due to a lack of knowledge about the phenomenon, indicators of abuse, and the procedures to follow when one becomes aware of such an issue. As a result, a great need has been identified for ongoing and updated training regarding more precise indicators of abuse and the procedures for the mandatory reporting of this phenomenon to the health department and to judicial authorities.

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Elder Abuse, Elder Abuse Prevention, Elder Abuse Protocol

I15 Explorative Study on the Level of Online Sexual Activities and Sexual Paraphilias

Cinzia Gimelli, PsyD, PhD, Science & Method, Via Regina Margherita 9/d, Reggio Emilia 42124, ITALY; Melania Lugli, PhD, Viale Montegrappa 29/C, Reggio Emilia 42121, ITALY; Davide Dèttore, PsyD, PhD, Viale Gaetano Pieraccini, 24, Florence, AL 50139, ITALY; and Andrea Giannelli, PHD, Università di Firenze, Viale Pieraccini, 6, Florence 50139, ITALY*

The goal of this presentation is to analyze the relationship between the level of Online Sexual Activities (OSA) and the presence of sexual paraphilias in a sample of 300 internet users (230 males, 63 females, and 7 transsexuals) between 18 and 58 years of age.

This presentation will impact the forensic science community by making attendees aware that the publicity about online "predators" who prey on naive children using trickery and violence is largely inaccurate.

Internet sex crimes involving adults and juveniles more often fit a model of statutory rape. Adult offenders who meet, develop relationships with, and openly seduce underage teenagers usually fit a model of forcible sexual assault or pedophilic child molesting. This is a serious problem, but one that requires different approaches from current prevention messages emphasizing parental control and the dangers of divulging personal information. Developmentally appropriate prevention strategies that target youths directly and acknowledge normal adolescent interests in romance and sex are needed. These prevention strategies should provide younger adolescents with awareness and avoidance skills, while educating older youths about the pitfalls of sexual relationships with adults and their criminal nature. Particular attention should be paid to higher-risk youths, including those with histories of sexual abuse, sexual orientation concerns, and patterns of offline and online risk taking. Mental health practitioners need information about the dynamics of this crisis and the characteristics of victims and offenders because they are likely to encounter related issues in a variety of contexts.

Purpose: Participant recruitment and data gathering were managed online through a self-report electronic questionnaire including the Internet Sex Screening Test (ISST), to assess the level of OSA and to divide the users into recreative users and at-risk users, and an ad hoc questionnaire (QTSPoo) elaborated to survey the presence of online and offline sexual paraphilias.

This survey represents an attempt to systematically analyze the phenomenon of online sexual paraphilias in comparison with the level of online sexual activity, already underlined by other clinical cases and studies.[1-5]

Internet paraphilias are a growing phenomenon, with a remarkable social impact. This is a complex phenomenon that was influenced, on one hand by the possibilities offered on the internet to those people who have such sexual interests and, on the other hand, by the ability to attract people who did not previously have such interests.[6-8]

Future research should be focused on the development of diagnostic criteria able to correctly determine the sexual disorder characterized by an excessive use of the internet (cybersex addiction or online sexual compulsivity) and to individualize the risk factors involved in this disorder, such as internet sex crimes against children.

The planning of research and its tools regarding this subject collides with the continuous technological evolution, in which it is difficult to fully predict future changes in the expression of virtual sexuality, which is becoming more complex and variegated both in expression and in ways in obtaining sexual satisfaction.

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Cybersex, Cybercrime, Sex Crimes Against Children

I16 The Assessment, Treatment, and Community Management of Sex Offenders

R. Gregg Dwyer, MD, EdD, Medical University of South Carolina, Community & Public Safety Psychiatry Division, 29-C Leinbach Drive, Charleston, SC 29407; J. Paul Fedoroff, MD*, Royal Ottawa Hospital, 1145 Carling Avenue, Ottawa, ON K1Z 7K4, CANADA; Lisa Murphy, MCA*, 1145 Carling Avenue, Sexual Behaviours Clinic, Ottawa, ON K1H 8N7, CANADA; Rebekah Ranger, BA*, University of Ottawa, Institute of Mental Health Research, 1145 Carling Avenue, Office 5463, Ottowa, ON K1Z 7K4, CANADA; and Natasha M. Knack, BA*, University of Ottawa, Institute of Mental Health Research, 1145 Carling Avenue, Office 5463, Ottawa, ON K1Z 7K4, CANADA*

The goals of this presentation are to: (1) provide practical and effective strategies for the assessment of sexual offenders and people with problematic sexual interests; (2) provide an overview of the current treatment strategies used among sexual offenders and people with problematic sexual interests; and, (3) provide an understanding of the similarities and differences between Canadian and American approaches to the assessment, treatment, and community-based management of this population.

This presentation will impact the forensic science community by providing information regarding the paraphilic interests and sexual offending behaviors from a forensic psychiatry perspective. The forensic science community will learn about the components of sexual offender assessment, the historical development of treatment models, evidence that treatment of paraphilias does work, and what measures are in place for community-based management of sexual offenders once they are released. Both Canadian and American approaches will be discussed.

This panel will provide an overview of Canadian and American perspectives on current methods used to assess, treat, and manage sexual offenders and people with problematic sexual interests. First, an overview will be provided of the assessment protocol for sexual offenders from the American perspective. The use of a tripartite approach with the components being clinical review, psychological instruments, and physiological assessments will serve as the framework. The physiological element includes visual reaction time measurement, Penile Plethysmography (PPG), and polygraphy. This will include a discussion of the development of an innovative phallometric stimuli set, as well as preliminary empirical data from an on-going study on this new set.

The Canadian perspective of the assessment protocol for sexual offenders will then be explored. Topics discussed include: referral sources, patient characteristics, and objective measures of sexual arousal as well as psychological measures that are currently being used in the Sexual Behaviours Clinic (SBC) of Royal Ottawa Mental Health Centre (The Royal) in Ottawa, Canada. New and innovative research projects will also be discussed, including objective assessment of female sexual arousal, which is in a pilot stage of study. Alternate and complimentary methods for the objective assessment of sexual arousal will be presented, such as **functional Magnetic Resonance Imaging** (fMRI) testing, eye-tracking, and new stimulus sets to be used during penile plethysmography.

Treatment strategies utilized between the 1960s and the present day will be highlighted. This will include the progression from solely behavioral techniques, such as aversion therapy, to more advanced and appropriate treatment interventions such as pharmacotherapy, cognitive-behavioral therapy, relapse prevention, and the Good Lives Model. Dynamic risk factors that are commonly targeted when treating this population and how these treatment targets have expanded and evolved throughout the years will be outlined. An overview of treatment strategies for special populations of sex offenders, such as juveniles, females, and individuals with intellectual disabilities, will be included in order to highlight the similarities and differences that are relevant when treating these various subgroups.

Next, evidence will be reviewed that challenges the hypothesis that paraphilic disorders are untreatable. Four perspectives that have been used to describe and treat the paraphilias will be explored. Evidence will be presented that supports the hypothesis that sexual interest can not only be controlled but changed. Explanations for why it may be time for a new paradigm regarding the effectiveness of treatment for men with paraphilias are covered.

This presentation will conclude with a review and discussion of the management of sex offenders in community settings, notably the use of Sex Offender Registries (SORs) and Public Notification (PN). The rationale for the use of this legislation and ethical concerns regarding the use of this type of community-based management will be explored. Academic research findings on the utility of these tools have largely been limited and inconsistent where available. Varying features of SORs across state and national lines has severely limited the ability for cross-sectional comparisons and broad legislative improvements. Legislation governing the international use of SORs and PN will be compared.

Sex Offender, Paraphilia, Treatment

I17 Autoeroticism in Autism Spectrum

Denise C. Kellaher, DC Forensics, Inc, 1750 Prairie City Road, #130-110, Folsom, CA 95630*

After attending this presentation, attendees will learn about potential sensory motivations in the autoerotic sexual behaviors in individuals with Autism Spectrum Disorders (ASD). Attendees will receive a summary of relevant research on sensory functioning and sexual behavior in ASD.

This presentation will impact the forensic science community by enhancing competence in performing psychosexual evaluations in both clinical and forensic contexts involving individuals with ASD.

Could sensory fascinations and core social skills deficits predispose ASD individuals toward autoerotic types of paraphilic disorders? Sensory response abnormalities are now included among the *Diagnostic and Statistical Manual-Fifth Edition* (*DSM-5TM*) criteria for Autism Spectrum Disorder (ASD), but little is known on how sensory issues impact the sexual behavior in this population. Hyper- or hypo-reactivity to sensory stimuli and unusual interest in sensory aspects of the environment may possibly manifest in restricted, repetitive patterns of sexual interests and/or sexual masturbation rituals that qualify as paraphilic behavior. That is, sensory sensitivities or, in some cases, insensitivities could explain paraphilic behavior among some ASD individuals.

The ASD adolescent may not experience masturbation exactly like the non-ASD adolescent, or so-called neurotypical, does. The ASD adolescent may struggle with either or both gross and/or fine motor skills such that he/she will use objects to better grasp, bind, or apply pressure to genital areas for the purpose of sexual gratification. Perhaps more significantly as it pertains to sex and sensuality, the ASD adolescent may be drawn to specific tactile, olfactory, other sensory, and even painful stimuli because of atypical proclivities. Sensory-specific masturbatory rituals in adolescence may crystallize into autoerotic or solitary and object-oriented paraphilic disorders in young adulthood. Autoerotic-type paraphilic disorders include Fetishistic Disorder, Transvestic Disorder, and Sexual Masochism Disorder. Published cases suggest that some ASD individuals may develop an autoerotic type of paraphilia associated with atypical sensory interests. Clinical cases outside of forensic contexts have emerged more frequently as well. In one case, an adult ASD male with Sexual Masochism Disorder experienced pinpricks and being bound tightly as pleasurable and necessary for sexual climax. When he was a teenager, he incidentally discovered that he liked pain-inducing stimuli and deep tactile pressure during masturbation while in the shower when he would shave with a straight razor around his genitals. He used a bathrobe belt to tie around his penis to pull it away from his body as he shaved himself cleanly and this is how binding became a necessary component to his masturbation ritual. He understood his hyposensitivity issues and sought noxious stimuli because without it he could not experience sexual satisfaction even with a partner.

Uncovering and clarifying behavioral motivations in both clinical and forensic contexts can make a difference in tailoring treatment recommendations and in making risk assessments, especially among individuals with ASD who may be more vulnerable to treatment failure, marginalization, stigma, and/or punitive treatment by others. This presentation will provide a review of published cases of ASD individuals with autoerotic type paraphilic behaviors with focused discussion on sensory motivations and relevant research on sensory abnormalities in ASD.

Autism Spectrum, Sensory, Paraphilia

I18 A Scientist-Practitioner Model for the Identification and Interpretation of Sadistic Offenders

Julian C.W. Boon, PhD, University of Leicester, School of Psychology, University Road, Leicester, Leicestershire LE1 7RH, UNITED KINGDOM; and Lynsey F. Gozna, PhD, University of Nottingham, Centre for Forensic and Family Psychology, Division of Psychiatry and Applied Psychology, Nottingham, Nottinghamshire NG8 1BB, UNITED KINGDOM*

After attending this presentation, attendees will better understand the distinctions between pseudo-sadism/BDSM, sadism, and other paraphilic interests and the implications for investigation.

This presentation will impact the forensic science community by assisting practitioners in the discernment of pseudo-sadism/BDSM from both sadism and necrophilia. Furthermore, attendees will have the opportunity, in session and beyond, for questioning and understanding the psychological and investigative implications of elucidating these distinctions.

A multi-factor, practitioner-oriented, working model of sadistic offenders is presented which has been developed from forensic clinical casework and the extant research literature. The model articulates the means of identifying the etiology of individual offender's sadism both in idiographic and nomothetic terms. It is argued that the identification of these aspects of sadistic offenders' personalities and their individual personality development histories are prerequisite factors to understanding the offenders and, accordingly, effective case management and the accurate risk assessment of recidivism.

Additionally, the model identifies the different forms of sadism — especially in distinguishing sexually sadistic from non-sexually sadistic offenders — articulating their significance for case management and risk assessment. The importance and means of distinguishing ego-syntonic sadistic offenders from those who are ego-dystonic are also featured in the model with comparable implications articulated. A further aspect which is addressed in this multi-factor practitioner model is the etiological link between sadism and necrophilia. In this regard, the forensic potential for understanding the possible overlap and the nature of distinct differences between those two psychological conditions is highlighted.

The model, rooted in clinical experience and case histories, also articulates the distinction between sadism cases and those that are rooted in pseudo-sadism/BDSM. It is argued that distinguishing between these two very different case backgrounds is critical for psychological understanding and case management, court assessment of mens rea, and risk assessment. BDSM-active couples, it is argued, are congruent in joint sexual/seemingly non-sexual enterprise and thus both parties have their mutual needs met. Other than "play acting," they are parties who are mutual in their activities.

In contrast, sadistic offenders are, ipso facto, authentic in their motivational orientation of their excitement through the expression of genuine fear in their victims. It is argued that this contrast is neither merely academic nor a nicety in regard to the forensic process; instead it is fundamental to establishing whether the alleged "victim" is the focus of a sadistic offender or whether he/she is complicit in the relationship with malicious intent.

Recommendations for forensic-related interagency cooperation and continued inter-communication in cases of sadistic offenders are discussed.

Sadism, BDSM, Investigation

I19 Interpreting and Assessing Benign and Malign Sexual and Non-Sexual Necrophilous Interests in Criminal Cases

Lynsey F. Gozna, PhD, University of Nottingham, Centre for Forensic and Family Psychology, Division of Psychiatry and Applied Psychology, Nottingham, Nottinghamshire NG8 1BB, UNITED KINGDOM; and Julian C.W. Boon, PhD, University of Leicester, School of Psychology, University Road, Leicester, Leicestershire LE1 7RH, UNITED KINGDOM*

After attending this presentation, attendees will understand and be able to articulate critical distinctions across cases in which individuals engage in necrophilous acts, including those causing harm (i.e., broader behaviors/interests relevant to a thematic understanding of such interest). This includes an awareness of personality- and lifestyle-relevant indicators, paraphilic-relevant interests (i.e., somnophilia), relationships (pseudo vs. genuine), and indicators of potential terrorist activity.

This presentation will impact the forensic science community by developing an understanding of the previously rarely recognized breadth of necrophilous activities and the ways in which these manifest in offending behavior within a wider paradigm of paraphilic interest and victimization. This will be applicable to criminal investigation, interrogation, community management, and therapeutic interventions in correctional facilities. The identification of the subtypes of necrophilous interest will be readily applicable for practitioners to implement into their work in forensic settings.

This presentation considers and discusses a personality-led approach to the development of malign and benign sexual and non-sexual necrophilous interests to differentiate elements between the subtypes and the implications for understanding and responding to such behavior in applied multi-disciplinary forensic settings. Hence, the focus is broadened from purely considering such acts as the sexual desecration of corpses and widens the understanding incorporating a personality perspective and red flags for the assessment of risk.

The intricacies of the behavior displayed by individuals who present with an interest in the more extreme aspects of negativity — necrophilous in theme — can be challenging to interpret and assess from the perspective of police investigation, case management, and therapeutic intervention. Due to the often-secret or isolated subculture of individuals who engage in necrophilous activities, criminal and non-criminal, it is important to consider further the etiology and development of extreme negative interests. Case examples will be presented to illustrate the variations in focus of the behavior and the contingent risk in addition to the factors that can indicate an increase in potential harm. In particular, the functional links between necrophilous and broader paraphilic interests are identified and it is argued that there are a range of strategies used — interpersonal and physical — to evade detection or exposure of their dark interests and risks. The challenges for practitioners working with such cases will be discussed in regard to the presentation of individuals and the link to mental health assessment, sexual offence risk, and public protection arrangements.

The presentation will conclude with focus on a discussion of the practice-based implications for professionals who engage with individuals who are drawn to negativity and, in particular, sexual and non-sexual necrophilous acts with consideration given to motive, mindset, and risk.

Necrophilous, Risk, Negativity

I20 Effects of a Treatment Program for Combat Veterans Charged With Domestic Violence

Giuseppe Troccoli, MD, Department Pathological Dependence, Via Putignani 228, Bari 70122, ITALY; and Mary Sullivan, MSN*, 4553 E Buist Avenue, Phoenix, AZ 85044*

After attending this presentation, attendees will better understand the effects of combat/war-related trauma as it relates to domestic issues and relationships, as well as on the therapeutic interventions targeted at treating the clinical and psychological consequences of such trauma and their impact on relationships back home. Furthermore, the specialized treatment programs for Domestic Violence (DV) perpetrators have been considered effective in preventing violent behavior in domestic situations and will result in valid applications for both clinical and forensic purposes.

This presentation will impact the forensic science community by outlining the factors and correlations that could influence the outcome of a program for combat veterans charged with DV.

Introduction: DV charges include a broad spectrum of different offenses. For example, behaviors associated with DV include fighting with someone within the domicile, use of weapons in the home, sexual assault, threatening to harm, and breaking into your own apartment after being evicted by your significant other. In Arizona, the consequences for these charges are significant, bringing drastic changes in the perpetrators' lives. For example, one may not be able to find employment or could be terminated from their current job, usually ending up homeless. Many studies confirm the high prevalence of DV among combat veterans, leading to the assumption that combat exposure could play a relevant role in the behavior of veterans coming home to their significant others.

In addition, co-variables of mental illness, substance abuse, and homelessness are known to be highly predictive of an increased risk of suicide. Therefore, entering a program will be a positive intervention in all variables. Substance abuse should be addressed during the program, but only as it impacts and complicates the issue of domestic violence in itself.

Method: During August 2008 and March 2012, 255 veterans were admitted into a DV treatment program in a Phoenix, AZ, medical center. Of the 255 veterans, 135 (53%) were Operation Enduring Freedom-Operation Iraqi Freedom veterans. All were court ordered to complete either a 26- (191 veterans), 36- (54 veterans), or 52- (10 veterans) week program, based on the charge(s). After an initial assessment (a 1:1 intake conducted by a forensic Registered Nurse (RN), which included a brief history of military service, a review of charges by the court/police report; a self-report by the veteran of what had occurred, a Dangerousness Assessment/Risk for Violence self-report of physical injuries, a brief orientation of what was to be expected of the veteran if accepted, and a signed agreement by the veteran), the program was based on individual psychotherapy (Cognitive Behavior Therapy (CBT) and group therapy. The forensic RN serves as a reporting authority to the probation office and to judges if the veteran is non-compliant with the rules of the program.

Results: The program was not completed by 31 of the veterans for the following reasons: 2 had charges dropped, 16 dropped out or showed non-compliance, 10 went to jail, 2 passed away before the program ended, and the judge would not allow 1 veteran to come to a program.

At some point after graduation, ten re-offended and were court-ordered to repeat the program (3.92% recidivism rate). Their general attitudes were documented and over time could be seen to be noticeably changed within their 26 or 36 weeks in the program, to the point that many did not want to leave. Many were sent for evaluation for Traumatic Brain injury (TBI) or for Post-Traumatic Stress Disorder (PTSD) treatment. Typically, the behaviors/symptoms/diagnoses presented included anger/hostility (with disregard for authority figures and women), insomnia and/or nightmares, paranoia, generalized anxiety disorder, panic attacks, amphetamine abuse, alcoholism, depression, suicidal thoughts, isolation of self, and bereavement in young men in their 20s to early 30s.

Conclusions: Specific correlations were found between trauma exposure and the clinical appearance of disorders. Generally, there were no significant outcome differences between long- and short-term treatment, suggesting a key role played by personality traits and motivation to follow the program.

A relevant factor that was recognized in a positive influence on outcome was represented by the quality of interventions, both regarding the personnel (competency, high experience, and ability of empathy) but also the specificity of the program that was reworked to accommodate veteran therapeutic needs. The benefits of the treatment could be confirmed by the registered low rate of recidivism after completion of the program.

Combat Veterans, Domestic Violence, Treatment Program

I21 The Psychopathy Checklist-Revised (PCL-R) Use for Psychopath Diagnosis — A Study of a Sample of Italian Female Offenders Deemed a Danger to Society

Felice F. Carabellese, MD, University of Bari, Section of Forensic Psychiatry, p.za G. Cesare, 11, Bari 70124, ITALY; Andrea Pinotti, MD, OPG Castiglione delle Stiviere, ASL Mantova, Mantova, ITALY; Donatella La Tegola, PhD, University of Bari, p.za G. Cesare, 11, Bari 70124, ITALY; Ilaria Rossetto, MD, OPG, Castiglione delle Stiviere, MN, ITALY; Filippo Franconi, MD, Delle Stiviere, Lonato, ITALY; Rosa Taratufolo, MD, p.za G. Cesare, Bari 70124, ITALY; and Roberto Catanesi, MD, p.za G. Cesare, Bari 70124, ITALY*

After attending this presentation, attendees will recognize characteristics of Italian female offenders deemed a danger to society.

This presentation will impact the forensic science community by identifying any psychopathological and phenotypic gender-specific factors related to psychopathy.

In Italy, the treatment of mentally ill offenders found not guilty (or partially guilty) by reason of insanity and at risk for recidivism ("a danger to society") is entrusted to the Judicial Psychiatric Hospital (OPG). The OPG is a high-security hospital, directly managed for more than 100 years by the Department of Justice and at present managed by the Department of Health. Their cultural and treatment profile has been maintained even after the reform law on assistance to the mentally ill in 1978, which in Italy determined the closure of psychiatric hospitals and the establishment of a community psychiatric-assistance model; however, a recent law ratified the closure of the OPGs, scheduled for March 31, 2015, and planned the transfer of the patients from the OPGs to community facilities located in their own regions. The six Italian OPGs accommodated approximately 1,500 patients, mostly men. The OPG of Castiglione delle Stiviere in northern Italy is the only one that admitted women; all women who have committed a crime on Italian territory and are at risk for recidivism are sent to the OPG of Castiglione. From this unique trait of the Castiglione OPG, came the idea for research on possible gender-specific factors related to psychopathy. The lower prevalence of psychopathy in women than in men may be due to several causes: sampling errors; errors related to gender differences in assessment tools; and differences in the phenotypic expression of antisocial behavior due to biological, cultural, and social variables. Several studies have reported a significant correlation, more prevalent in women than in men, between psychopathy and Borderline Personality Disorder (BPD). It has been suggested that BPD in women represents the phenotypic expression of psychopathy.

This study was conducted in the OPG of Castiglione delle Stiviere from February 1, 2013, to the end of 2013. At that time, the population of the OPG women's section was composed of 86 women (men's section=230m).

The main purpose of this research was to identify any psychopathological and phenotypic gender-specific factors related to psychopathy.

Method: The survey was conducted in regard to the rules considered by the ethics committee of the structure. This study subjected all 86 women, who had given their consent, to clinical and anamnestic evaluations. This study administered SCID I and II interviews and other mental tests (MMPI-2, MCMI-III, R-Bans) to the entire sample, after a period of observation. A clinical-anamnestic assessment was performed to investigate age, marital status, education, personal and family psychiatric history, legal status (infirmity/partial infirmity), type of crime (property/person), and pharmacological therapy. To evaluate the index of psychopathy, the Hare Psychopathy Checklist-Revised (PCL-R) was used. In this research, a score of ≥25(the recognized European cut-off level) was considered indicative of psychopathy.

Psychopathy, Women Offenders, Gender-Specific Factors

I22 From Forensic Sciences to the Stars: Study for the Implementation of a Protocol to Protect Astronauts Based on an Evaluation of Criminal Trials and Behavioral Genetics

Vincenzo Lusa, JD, Via Ferdinando, Palasciano #72, Rome 00151, ITALY; and Annarita Franza, PhD*, Via Delle Oche 15, Florence, ITALY*

After attending this presentation, attendees will better understand the importance of recent studies on behavioral genetics drawn from Italian criminal trials in which the defendants were found to have polymorphisms and brain abnormalities capable of predisposing deviance, assuming primary importance when they are applied to an area such as space exploration.

This presentation will impact the forensic science community by: (1) acquainting lawyers and forensic scientists of the benefits related to the creation of security procedures designed to safeguard humans on missions in which they will be isolated and in confined areas for long periods of time; and, (2) being able to identify both the types of biomarkers predictive of human behavior as well as the structural and functional abnormalities of those parts of the brain that promote criminal acts.

Since 2009 in Italy, some individuals (even some without criminal records) have been prosecuted who, although convicted of murder, benefitted from reduced sentences through the verification of some genetic polymorphisms and **Computed Axial Tomography (**CAT), **Positron Emission Tomography (**PET), and **functional Magnetic Resonance Imaging** (fMRI) results which showed brain malformations that may produce manifestations of violence.

The defendants in the trials were Bayout (Trieste court, 2010), Albertani (Cremona court, 2012), Mattielo (Venice court, 2013), and Gatto (Catanzaro court, 2013). In the first case, the offender responded to severe environmental stress. In the second and third cases, the defendants, with no prior criminal records, unexpectedly killed family members. In the last case, the offender acted emotionally in a crime of passion. The presence of biomarkers predictive of deviance in human behavior and significant anatomical abnormalities of the brain were ascertained in all the cases. The murders in question were committed because the defendants suddenly experienced manifestations of stress and environmental effects, in spite of being in full possession of their faculties.

This demonstrates the importance of developing a psychological protocol to evaluate the aspiring astronaut's genetic background in relation to the environment in which he/she will operate. In fact, the behavior of people working far from Earth is usually affected by high levels of stress, similar to what astronauts are often subjected to. Among the space travel risk parameters evaluated by the National Aeronautics and Space Administration (NASA) are behavioral health, bone metabolism and physiology, nutrition, immunology, cardiac and pulmonary physiology, space radiation, and space human factors. Moreover, NASA has identified three categories of behavioral health and performance risks associated with long-duration spaceflight and exploration: (1) adverse behavioral conditions and psychiatric disorders; (2) performance of errors resulting from sleep loss, circadian desynchronization, extended wakefulness, and work overload; and, (3) performance reduction due to inadequate cooperation, coordination, communication, and psychosocial adaptation within a Team Gap.[1] Billica reported a 2.86 per person-year incidence of such problems among the 508 crew members who flew on 89 space shuttle missions between 1981 and 1989.[2] The most common behavioral symptoms reported by crew members were anxiety and irritability. Data collected for 28.84 person-years of NASA space flight identified 24 cases of anxiety, for an incidence rate of 0.832 cases per person-year.[3]

Discussion will illustrate how security procedures to protect astronauts can be implemented and which genetic tests and diagnostic actions are suitable to studying the essential parameters under which biological tests on candidates will also be carried out to identify some polymorphisms such as Monoamine Oxidase A (MAOA), serotonin receptors 5-HTRIA and 5-HTR1B, serotonin transporter SLC6A4, Tryptophan Hydroxylase 1 and 2 (TPH1 and TPH2), the Catechol-O-Methyl Transferase (COMT) gene, human dopamine receptor DRD4, the DBH gene, the Androgen Receptor (AR), and the Estrogen Receptor (ESR1).

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NASA, Neuroscience, Behavioral Science

I23 Suicide Note Writers: Are Medicolegal and Forensic Psychiatric Items Linked?

Pasquale Beltempo, MD, D.I.M, Sezione di Medicina Legale, Piazza Giulio Cesare, 11, Bari, Puglia 70124, ITALY; Ilaria De Vitis, MD*, Via Carducci 23, Cavallino (le) 73020, ITALY; and Roberto Catanesi, MD, p.za G. Cesare, Bari 70124, ITALY*

The goal of this presentation is to evaluate the correlation between medicolegal and forensic psychiatric aspects of suicide. Although numerous studies have been published on suicide, relatively few have systematically examined suicide notes as a source of data about these events from a multidisciplinary approach.

This presentation will impact the forensic science community by underlining the role of suicidal reports as a valuable source to acquire important information about the state of mind of decedents in the time period prior to their deaths.

Some studies have found differences between suicide note writers and those who do not leave notes; it is therefore argued that information contained in suicide notes may not be generalizable to suicide decedents who do not leave notes. A retrospective study was conducted based on nearly two decades of external examination and crime scene inspection reports performed by the residents of the Institute of Legal Medicine of the University of Bari. A total of 249 suicide cases were reported between 1997 and 2015. In 48 cases (19.3%), one or more suicide notes were found during crime scene inspection or were reported by police officers assisting the residents during external examination. Suicide notes primarily consisted of handwritten letters (97.59%). In 39 cases, the suicide note writer was male, while in only nine cases were notes written by female decedents. These percentages were similar to those observed in the non-writers population (74.12% male, 25.87% female). The average age was 51.82 years in the note writers and 52.63 years of age in the non-writers.

The reviewed data showed a substantial difference in the methods used to commit suicide. The leading methods in suicide note writers were hanging (47.9%), gunshot wounds (18.75%), and falls from height (14.89%). In the non-writer population, falls from height were first (37.81%), followed by hanging (31.34%), and gunshot wounds (12.94%). The leading location where suicide was committed in both groups (58.33% of the writers group, 59.20% of the non-writers) was at home. A psychiatric condition, mostly depression, was reported for 22 of the 48 suicide victims who left notes (45.81%) and in the 46.76% of the population of non-writers. The suicide notes were analyzed based on content, according to scientific literature on the subject. In the group of suicide note writers with no diagnosed psychiatric condition, the leading content of the message was justification of the act (30.43%), followed by forgiveness (21.74%), farewell, instructions, and confirmation of the suicide (13.04% for each category, respectively). Anger was expressed in only one case of this subgroup, left by a father who accused his heirs of their behavior. This study reports a case of a 16-year-old boy who was bullied for being “feminine” and who hung himself, leaving two pages of handwritten instructions concerning his funeral.

In the subgroup of suicide note writers with a history of a psychiatric condition, the content of the messages was forgiveness (32.36%), justification (29.03%), and instructions (12.90%). In one case, the message, a series of numbers, was written on the hand of the decedent, a 42-year-old female with a diagnosis of bipolar disorder who was found hanging in the psychiatric ward. The analysis of the collected data showed percentages that were similar to those reported in previous studies.

The most significant data emerging from this research concerns the correlation between the suicidal method chosen in the note-writer group and in the non-writer group. It can be hypothesized that the note writing and the suicidal method are linked from a behavioral point-of-view: note writers tend to keep the suicide scene and the place where they leave the note close together, thereby avoiding suicidal methods that separate the decedent from the message. As a matter of fact, a suicide note was left in 7.69% of the railway suicides and in 8.43% of the falls from height. Furthermore, it has been observed that methods with a greater chance of suicidal plan failure are chosen by note writers: it can be speculated that note writers consider the possibility to exploit the failure of the method as a demonstrative act.

Suicide Note, Suicide Method, Multidisciplinary

I24 Stalking Charges Among Defendants Referred for Competency to Stand Trial and Criminal Responsibility Evaluations: A 10-Year Case Series

Christopher Fields, MD, Medical University of South Carolina, 29 C Leinbach Drive, Charleston, SC 29407; Sheresa Christopher, PhD*, Medical University of South Carolina, 29 Leinbach Drive, Charleston, SC 29407; Diana Mullis, MD*, Medical University of South Carolina, 29-C Leinbach Drive, Charleston, SC 29407; and Adam Bloom, MD*, Medical University of South Carolina, 27-C Leinbach Drive, Charleston, SC 29407*

After attending this presentation, attendees will: (1) be familiar with common typologies of stalkers advanced in the current literature; (2) understand the risks currently associated with stalkers of formerly intimate partners and psychotic stalkers; and, (3) become familiar with appropriate intervention measures for stalking perpetrators.

This presentation will impact the forensic science community by providing a review of current stalking literature, including epidemiogical data, motivation of perpetrators, typologies of stalkers, and potential interventions for perpetrators. By focusing on a forensic sample referred for competency and criminal responsibility evaluations, attendees will gain a better understanding of the mindset of individuals charged with the most serious cases of stalking, those necessitating significant legal intervention.

Since 1990 when stalking was criminalized in California, there has been increasing interest in this complex phenomena.[1] Multiple definitions of stalking have been put forth with most indicating that a pattern of unwanted pursuit must exist followed by a perceived threat to the safety of the victim resulting in the induction of fear within the victim.[2] Stalking research has involved the investigation of multiple related factors, among them: epidemiology, psychopathology, motivation, typology, violence risk, victim impact, and neurobiology.[3] These studies have examined diverse populations of stalkers including clinical samples, forensic referrals, court-referred individuals, and community-based populations.[4,5]

The current study targets the review of forensic psychiatric records of pretrial detainees accused of stalking or aggravated stalking who were referred to an outpatient forensic program for Competency to Stand Trial (CST) and Criminal Responsibility (CR) evaluations in the past ten years. The study examined these outpatient forensic referrals in the context of typologies within the stalking literature encompassing psychotic, non-psychotic, acquaintance, stranger, and intimate stalking scenarios. The data were analyzed to identify primary psychiatric diagnoses, personality diagnoses, existence of psychopathy, and patterns of behavior that were potentially associated with the likelihood of being found incompetent, not criminally responsible, or impacting the risk for violence. Using a case series, the findings differences in traits among stalking defendants deemed competent, not competent, criminally responsible, and insane are described. Correlational research methods will be utilized to delineate the relationships among the various variables described. Implications of the data assessed will be discussed to stimulate future research directions.

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Stalking, Psychiatry, Competency

I25 The Sound of Music: Effects on Post-Traumatic Stress Disorder (PTSD)

Sundeep S. Randhawa, MD, Columbia/Cornell University, 640 W 170th Street, Apt 5C, New York, NY 10032; and Michael Liepman, MD, Western Michigan Homer Stryker M.D. SOM, 1717 Schaffer Street, Ste 010, Kalamazoo, MI 49048*

After attending this presentation, attendees will be familiar with PTSD with delayed expression and dissociative symptoms treated via pharmacotherapy, psychotherapy, and music therapy. Attendees will better understand the use of non-pharmacotherapy options such as music therapy with difficult-to-treat patients. The music being used will be explained in detail along with the therapeutic relief it provided. Attendees will understand how someone accused of pedophilia, behind bars and many miles away, can continue to induce such impairing PTSD-related symptoms in others.

This presentation will impact the forensic science community by increasing awareness of the continual power a pedophile behind bars can have over his/her victim. Additionally, this presentation will demonstrate how factors such as the specific location and security level of a prison in which the pedophile resides can directly influence a victim of PTSD.

Introduction: PTSD is an impairing condition that was first documented during the Civil War as "irritable heart." The lifetime incidence is estimated to be 9% to 15% of the general population. These individuals have experienced, witnessed, or confronted a life-threatening event and become significantly impaired in their activities of daily living. Pharmacotherapy and psychotherapy remain the mainstay treatment for PTSD; however, short-term modalities are also being utilized on a case-by-case basis.

Case Report: Lady R is a 54-year-old Caucasian female who was seen as an initial evaluation in the outpatient psychiatry clinic. She was referred by her primary care physician and psychologist with the chief complaint of, "I want to improve the noise in my head." During the evaluation, it was found that Lady R was leading a well-balanced life with her husband when suddenly things began changing after attending a court hearing 15 years prior in which her older brother was being accused of pedophilia. During this hearing, Lady R heard the testimony of the 4-year-old victim explaining what had happened along with hearing the things she was forced to say during the encounter. Throughout the next few years, Lady R began experiencing increased anxiety, irritation, frustration, nightmares, and variable moods but was unable to pinpoint the source. Dosages of clonazepam (1mg) and venlafaxine (37.5mg) were initiated by her primary physician and initially helped her anxiety and sustained depressive symptoms. Two years ago, Lady R went out for a glass of wine with a friend, who was a masseuse. It was an average night filled with conversation and laughter that culminated in Lady R receiving a cranial massage, then going home and to bed. Upon awakening, her husband approached her with caution asking if she remembered the previous night. He explained that when he tried to cuddle with her, she regressed into a fetal position and started crying out, "Please don't hurt me, please don't hurt me," in a voice pattern similar to a young girl, followed by, "Let her sleep, let her sleep," in a male voice when he attempted to wake her. Since this incident, Lady R experiences vivid flashbacks, avoids talking about her older brother, stopped working, is hypervigilant, and has non-stop "noise" in her head, as she explains it. Due to medical concerns, the accused brother has been transferred to a prison closer to her home, thus increasing her fear. Upon evaluation, treatment was started with supportive psychotherapy and her home medications (venlafaxine 150mg qday, bupropion 150mg BID, clonazepam 1mg qday, and quetiapine 25mg qhs) were continued. After several sessions, Lady R continued to be distressed by this "noise," despite psychotherapy/medications. Listening to music had been mentioned during the sessions as an alleviating factor for Lady R, which was then encouraged. Music therapy continued to alleviate daytime symptoms and was then prescribed for Lady R as adjunctive treatment with her continued psychotherapy and medications. Since the addition of active/passive music therapy, her symptoms have decreased and Lady R continues to improve her functioning both socially and at home.

Discussion: Being a victim of pedophilia can lead to lifelong symptoms of anxiety, mood, and depressive symptoms. Dealing with those symptoms can be challenging enough, so what additional treatment challenges arise when factors such as proximity to the perpetrator, treatment-resistant anxiety, and delayed-onset (40 years) PTSD are involved? Music therapy is one such treatment modality that can be beneficial. Active and passive listening to unfamiliar music can show increased symptom relief secondary to not having any linked memory to the song. Music is universal and can be utilized in any specialty for an individual experiencing a perceived stress.

Conclusion: PTSD is an impairing disorder that has a variable presentation with each case and can be very difficult to treat. The initial trauma can ruminate in the victim's mind and lead to symptoms of depression, anxiety, mania, and even psychosis. Pharmacotherapy along with continued psychotherapy is the treatment of choice, but new treatment strategies (i.e., music therapy) are also being utilized with added benefit. This patient found relief in music that was familiar and unfamiliar to her, which decreased the initial "noise" and led to improved functioning on a day-to-day basis despite the accused prisoner being closer to her in distance.

Treatment Resistant PTSD, Music Therapy, Dissociation

I26 Reducing the Risk of Violence in a Psychiatric Inpatient Setting by Examining External Factors

Rebecca Najera, DO, USC Institute of Psychiatry and Law, PO Box 86125, Los Angeles, CA 90086-0125*

After attending this presentation, attendees will better understand current studies that examine modifiable risk factors that may decrease violence in a psychiatric inpatient setting. In addition, alternate dynamic risk factors that may play a role in decreasing violence, based on record reviews and literature of assaults that analyzed these factors, will be discussed.

This presentation will impact the forensic science community by analyzing further what can be implemented to decrease assaults in a psychiatric inpatient setting. A review of the existing literature will provide information on important factors that may play a role in patient aggression and assaultive behavior.

Working in a psychiatric inpatient facility is not without its safety risks and must be approached with caution and acute awareness of one's surroundings at all times. As is well known, some psychiatric patients in an acute psychiatric hospital can be impulsive and violent and may strike out at staff or other patients while hospitalized. Due to this awareness, the implementation of violence risk assessments is now commonly used as a tool in identifying those patients who might be at risk for violence. Upon evaluation of these individuals on an initial intake, it is important to determine which patients should be placed on assaultive precautions and which should not. A number of violence risk assessments exist currently that attempt to classify these individuals as low, medium, or high risk for violence. The goal of this classification is to ultimately reduce assaults in a psychiatric hospital.

There is existing literature which examines the risk factors that can be the impetus for assaultive behavior among psychiatric patients. The most recent studies focus on risk stratification; however, studies that address changes in risk and subsequent interventions are limited. Some risk factors may be modifiable to reducing the occurrence of violent acts while hospitalized; others may not be. For example, a person who is intoxicated when first admitted may have a violence risk status that changes. On the other hand, if individuals' assaultive behaviors are linked to their personality disorders, then their risk for violence may not be modifiable during their hospitalization. With these individuals, other factors must be considered to decrease their propensity for assaultive behavior while on the inpatient psychiatric ward.

At the Los Angeles County University of Southern California Medical Center, assaults involving physical contact are noted in the center's intranet patient safety network. This serves as a means for tracking assaults and obtaining more details about the incident that occurred. The information was collected over a span of one year, from March 2014 to March of 2015, and resulted in a total of 48 logged assaults which will be presented.

In March of 2014, a violence risk assessment was devised and implemented into the initial intake process at the inpatient psychiatric facility. This tool combined risk factors taken from pre-existing violence risk assessments that were more commonly seen in this particular population of generally low-income patients. It was discovered through the record review that many of the patients were labeled as medium to high risk for violence. Some assaulted more than once while hospitalized. Further analyses will now be directed toward analyzing the data to determine if there were any modifiable risk factors to the inciting events with regard to: precipitating factors; medication non-compliance; primary treating provider (intern, second-year resident, attending); individual room or shared room; length of stay; and, ward size and milieu. A literature review will be conducted to determine if there are other modifiable variables that are successful in decreasing violence and will be included in the analysis.

Inpatient Assault, Violence, Modifiable Risk Factors

I27 The Impact of Child Abuse Charges for Prenatal Substance Use on the Medical Treatment of Pregnant Women With Opioid Use Disorders

Cara Angelotta, Columbia University, 501 E 75th Street, #7C, New York, NY 10021*

WITHDRAWN

I28 The Risk of Assault by Patients in Psychiatry Settings: A Case Report and Review of the Literature

Giancarlo Di Vella, MD, PhD, University of Torino, Dept Public Health Sciences, Sezione di Medicina Legale, Corso Galileo Galilei 22, Torino 10126, ITALY; Lucia Tattoli, PhD, Sezione di Medicina Legale, University of Turin - Corso Galileo Galilei, 22, Torino 10126, ITALY; Fiammetta Marella, Via Cassini 57, Torino 10129, ITALY; Mary Sullivan, MSN, 4553 E Buist Avenue, Phoenix, AZ 85044; Roberto Catanesi, MD, p.za G. Cesare, Bari 70124, ITALY; and Ignazio Grattagliano, PsyD, University of Bari, Piazza Cagnola, 3/B, Casamassima, Bari 70010, ITALY*

After attending this presentation, attendees will understand that assaults by psychiatric patients against mental health care providers is a significant occupational risk for health care staff in private and public acute psychiatric facilities and rehabilitation wards. The review of literature shows that aggressive behavior, in most cases, involves verbal aggression and that physicians and nurses reported the highest prevalence of violence. Several surveys revealed that younger patients (=25-30 years of age) with multiple diagnoses, including substance abuse, psychotic behavior, and non-compliance to treatment are at the greatest risk of violent behavior, without a great gender difference.

This presentation will impact the forensic science community by emphasizing that mental health professionals can become victims of lethal assault by psychiatric patients, with minor injuries being more common (i.e., resulting in missed days of work or assignments to limited duty). Multiple or life-threatening injuries (i.e., fractures, lacerations, bruises, or a loss of consciousness) are sustained by a smaller percentage of staff members.

A case of a 53-year-old female psychiatrist who was found stabbed in her office in a mental health center was reported. A 44-year-old male was charged with this fatal assault. He had been previously admitted to the hospital with suicidal ideation and confusion. A 14.5 centimeters-long kitchen knife (single cutting edge) was found in the office. The autopsy revealed 70 stab wounds: four superficial wounds of the supraclavicular and cervical areas; three abdominal wounds penetrating the liver; eight wounds of the thorax penetrating the lungs; forty-two wounds of the back (twenty-eight of which penetrated pleural cavities); six superficial wounds of the lumbar region; and seven superficial wounds of the upper arms. The same knife found in the crime scene caused all of the wounds. Death was attributed to massive hemorrhagic shock. The forensic psychiatric expert highlighted a borderline-antisocial personality disorder; the perpetrator was judged competent to stand trial and the prosecutor asked for 30 years in prison.

Results from the literature review indicate that patient aggression toward mental health care professionals is common and worldwide. These incidents raised the controversial debate regarding the potential danger posed by individuals with mental illness, as psychiatrists have a 5% to 48% chance of experiencing a physical assault by a patient during their career. According to the United States Department of Justice's National Crime Victimization Survey conducted from 1993 to 1999, the annual rate of non-fatal, job-related violent crime was 12.6 per 1,000 workers in all occupations. Among physicians, the rate was 16.2 per 1,000 and among nurses was 21.9 per 1,000; however, for psychiatrists and mental health care professionals, the rate was 68.2 per 1,000, and for mental health custodial workers the rate was 69 per 1,000.[1] The most common type of aggression has minor consequences (mostly psychological as symptoms suggestive of post-traumatic stress disorder) and has usually occurred in crowded and unstructured settings without weapons, but a few cases of serious injuries or death are reported.

Mental health care practitioners have to be aware that risk factors for violence are divided into two categories: static (psychiatric diagnoses of major mental illness and prior history, young adulthood, lower intelligence, history of head trauma or neurological impairment, dissociative states, history of military service, and weapons training) and dynamic (substance abuse or dependence, persecutory delusions, command hallucinations, treatment non-compliance, impulsivity, homicidality with a feasible homicidal plan, depression, hopelessness, suicidality, and access to weapons). A multidisciplinary continuing education curriculum focused on recognizing aggressive or violent behavior between mental health practitioners and their patients is of paramount importance for preventing violent assaults is suggested. The ability to recognize the key "warning signs" (psychomotor agitation, combative posturing, guardedness, paranoid remarks, low frustration tolerance, emotional lability, and irritability) that may precede violence will increase staff safety and may save the lives of all involved.

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Homicide, Psychiatric Safety, Violence

I29 Aggression and Harassment: An Underrated Risk in the Health Care Workplace

Ignazio Grattagliano, PsyD, University of Bari, Piazza Cagnola, 3/B, Casamassima, Bari 70010, ITALY; Stefano Berardi, MD, University of Bari, Piazza Giulio Cesare, Bari 70124, ITALY; Gabriella Martina, MD, University of Bari, Piazza Giulio Cesare, Bari 70124, ITALY; Antonio Baladassarre, MD, University of Bari, Piazza Giulio Cesare, Bari 70124, ITALY; Luigi Vimercati, MD, University of Bari, Piazza Giulio Cesare, Bari 70124, ITALY; and Marina Musti, MD, University of Bari, Piazza Giulio Cesare, Bari 70124, ITALY*

After attending this presentation, attendees will appreciate the importance and under-valuation of workplace violence in the health care setting.

This presentation will impact the forensic science community by demonstrating that violence occurring in a health care workplace environment is a serious, but unevaluated, problem that needs to be adequately addressed. Recommendations on how to confront this problem are suggested.

"Workplace violence" is defined by the National Institute of Occupational Safety and Health (NIOSH) as "violent acts (including physical assaults and threats of assaults) directed toward persons at work or on duty." Over the past years, the scientific community and the media have given significant attention to this phenomenon. It is estimated that about one-quarter of such violence takes place in the health care sector. Several studies are available in the literature that primarily concern aggression toward nurses, particularly emergency room nurses. This appears to be the most at-risk health care category.

However, the exact extent of this phenomenon is difficult to define, given the tendency to not report these incidents. Often they are considered to be "part of the job" and "inevitable." Violence against health care workers is present all over the world, in developing countries as well as industrialized ones. In one study that examined several countries (Brazil, Bulgaria, Lebanon, Portugal, South Africa, Thailand, and Australia), it was revealed that more than 50% of workers experienced at least one episode of physical or psychological violence in the 12 months preceding the survey.

Psychological violence is much more prevalent than physical violence, particularly "verbal abuse." The European Agency for Safety and Health at Work (EU-OSHA) reports that 15% of European health care workers experienced aggression over the course of 2010. In one American study involving 3,465 emergency room nurses, results showed that in the three years prior to the investigation, approximately 25% of the analyzed sample were victims of physical aggression more than 20 times, and almost 20% were the victims of 200 incidents of verbal abuse.

In Italy, there are still very few studies concerning violence directed at health care workers. Only in the last few years has there been more attention paid to this issue: in one investigation performed every two years in a local Italian health unit between 2005 and 2011, 9% of the 1,411 study participants affirmed that they had been the victims of stalking. Moreover, estimates regarding the frequency of physical aggression over a one-year period vary between 3% and more than 7%. It is even more difficult to evaluate the frequency of non-physical aggression that, according to several studies, is reported to involve between 38% and 90% of hospital workers.

For this reason, it is essential to monitor the issue of aggression, even through the use of incident-reporting forms. Not only violence perpetrated by patients but violence between workers must also be monitored using a standardized "reporting system" because such aggression represents a considerable source of work-related stress for hospital employees.

Objectives: Examine the problem of aggression and harassment in the context of community health according to the professional category and workplace. The goal is to identify the subjects and the work environments that are most at risk and to formulate prevention and coping strategies to address the problem (e.g., best practice, mitigation action, and listening centers).

Method: From one of the biggest hospitals in southern Italy, 107 workers (doctors, psychologists, nurses, auxiliary social workers, and social assistants) were recruited and 88 workers who have the same professional qualifications were recruited from local social-health care service centers.

Instruments: After having obtained consent from all of the workers, a semi-structured, detailed questionnaire was administered that contained personal and epidemiological data, as well as information related to harassment or violence by patients or their family members, colleagues, or superiors.

Final Considerations: Episodes of violence against health care workers may be considered sentinel events because they signal the presence of risk situations or vulnerability in the work environment that requires the adoption of appropriate prevention and protection measures for workers.

In order to prevent violence in hospitals, employers must develop a safety and health plan that includes commitment by management, worker participation, identification of danger, security and health education, risk prevention, monitoring, and incident reporting. Employers should periodically evaluate this plan.

Workplace Violence, Harassment, Health Care Workplace

I30 Genetic Study of Single Nucleotide Polymorphisms (SNPs) in the Oxytocin Receptor (OXTR)

Elizabeth Chesna, BS, 555 Bowers Boulevard, Apt 1424A, Huntsville, TX 77340; Gabriella Cansino, MS, 1717 Bald Eagle Drive, McKinney, TX 75071; Peyton Gandy, MSFS, 2052 Myrtle, Unit 3, Dover, DE 19901; Jessica Wells, MS, Department of Criminal Justice and Criminology, 816 17th Street, Huntsville, TX 77340; Danielle Boisvert, PhD, Department of Criminal Justice and Criminology, 816 17th Street, Huntsville, TX 77340; Todd Armstrong, PhD, Sam Houston State University, College of Criminal Justice, 816 17th Street, Huntsville, TX 77320; and David A. Gangitano, PhD, Sam Houston State University, 13906 Paradise Valley Drive, Houston, TX 77069*

After attending this presentation, attendees will better understand the relationship between SNPs in the OXTR and specific behavioral traits.

This presentation will impact the forensic science community by demonstrating that certain genotypes of OXTR SNPs are associated with specific behaviors. The imbalance of oxytocin and serotonin is also thought to be a crucial factor in psychopathy. Results from this study are consistent with previous research; however, this is the first reported study of genotypes in the OXTR and behavior of the main ethnic groups in the United States.

Oxytocin plays an important role in social behavior and is associated with empathy, bonding, and trust. Previous studies have found that both gene expression and environment impact the development of certain behaviors. Furthermore, behavior is influenced by the level of neurotransmitters and the expression of their receptors. The OXTR gene contains many polymorphic sites including SNPs. SNPs are single base-pair differences at a specific location within the genome. Other studies have found that DNA polymorphisms within the OXTR gene were linked with different traits such as psychopathy, aggression, and empathy. While genotypic differences between males and females have been reported, the influence of ethnic background still remains unknown. The purpose of this study was to determine the association between OXTR SNP genotypes and behavior. Moreover, this study also includes the analysis of gender and ethnic characteristics.

A student population from Sam Houston State University (*N*=527) was the target of this study. It consisted of the three main ethnic groups found in the United States: Caucasian, Hispanic, and African American. All samples were genotyped using four SNPs within the OXTR gene: rs11476, rs53576, rs6770632, and rs1042778. A survey was given to each participant to assess different types of behavior including aggression, empathy, and antisocial behavior that may be influenced by oxytocin.

All three populations studied showed no evident departures from Hardy-Weinberg equilibrium; however, significant departures were detected for linkage disequilibrium due to the close location of these markers within the OXTR gene. Statistically significant differences were observed among the three ethnic groups for allelic frequencies of rs6770632 and rs1042778. No gender differences were observed for any of the SNP markers. Using logistic regression, statistical differences were found for the Caucasian population in SNPs rs53576 and rs1042778 for both empathy and aggression. Evidence for association between aggression and SNP rs1042778 was observed in the Hispanic group. The OXTR SNP rs853576 revealed an association with empathy in both the Caucasian and African American population. The results indicate an association between OXTR SNPs and aggression and empathy that is dependent on ethnic background.

In conclusion, this study found genotypic differences between ethnic groups that resulted in phenotypic or behavioral differences. Furthermore, it was shown that OXTR polymorphisms in SNPs rs53576, rs1042778, and rs853576 affect behaviors such as aggression and empathy.

Behavioral Genetics, DNA Typing, Single Nucleotide Polymorphism

I31 Means and Dynamics of Suicide in Human History

Luca Massaro, MA, via degli Artigiani n° 4, Este 35042, ITALY; and Matteo Borrini, PhD*, Liverpool John Moores University, RCEAP-School of Natural Science & Psych, Byrom Street, Liverpool L3 3AF, UNITED KINGDOM*

After attending this presentation, attendees will understand the methods most commonly used to commit suicide, from both chronological and geographic-cultural perspectives.

This presentation will impact the forensic science community by proposing an operational criterion for crime scene investigations in cases of undetermined cause of death or suspected suicide.

In view of the multi-ethnicity of modern societies, an investigative standard could also be proposed in which the professional figure, a person technically expert in discriminating between typical and atypical suicide, has become essential.[1] It is now important to contextualize the choice of the method of suicide and the environment in which it took place within the cultural setting, geographic region of origin and/or residence, and traditions of the victim.[2] To these elements, personal characteristics, any psychiatric pathologies involved, the type of work (if any) in which the subject was engaged, etc., must be added. According to a combined interpretation (the theories of aggressivity and behavioral psychology), suicides (people who kill themselves) are those who have reached the point of "convincing" their victim (themselves) to put an end to their psychic pain and thus to their life. They then elaborate this idea and plan to commit suicide. Ideation and planning may sometimes also be simultaneous.

This study examines the differences between methods of suicide in time and space, in order to understand the reasons for them, and why different methods, painful or otherwise, various techniques and motives, and possible geographic or cultural trends exist. This historical and analytical study encountered three basic problems: (1) worldwide, there are now nearly one million suicides per year; in general, no specific forensic death investigation is conducted, but rather a classic forensic death investigation, to reveal any positive signs of homicide; (2) true forensic work only began about a century ago and has only used scientific methods for a similar period (not always the case in every country in the world); and, (3) many countries do not supply official data on their annual numbers of suicides.[3,4] This means that the exact dimensions of the phenomenon of suicide in the world are still underestimated. Although these limitations are clear-cut, they do not allow us to reach definite conclusions; however, it seems that the methods most frequently used over the centuries have been hanging, stabbing, and shooting.

In regard to dynamics (scene), *public* suicide, traditional in some ancient Far Eastern communities, has now taken on public forms in many other situations; they may be announced in public, filmed on social forums, or occur in public places.[5] In more modern times, the suicide of mass murderers or serial killers, suicides in prison, mass suicides, suicide pacts, suicides within particular communities living with others (e.g., native Americans, Canadians, Australians, and Tasmanians), suicide by imitation, adolescent suicide, and homicide-suicide are all typical. Another important aspect of international reports of suicide is the fact that suicide by terrorists was a phenomenon already occurring in the 20th century.[6]

In conclusion, this study suggests the need for a deeper knowledge of suicide, so that particular attention can be paid to crime scene analysis in cases of undetermined cause of death and proper examinations made from the medicolegal, psycho-criminological, and anthropological viewpoints. This approach allows the formulation — according to criteria of high statistical probability — of a *positive* diagnosis of suicide in cases of undetermined cause of death or suspected homicide, based on the *logic of exclusion*. This may be an investigative criterion, although not necessarily the only one.

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Suicide, Forensic Death Investigation, Typical Suicide

I32 Neurobiology of Psychopathy: Developments and Directions

Arin Abnoosian, MD, 249 N Brand Boulevard, #312, Glendale, CA 91203; and Michael Cummings, MD, California Department of State Hospitals, Patton State Hospital, 3102 E Highland Avenue, Patton, CA 92369*

After attending this presentation, attendees will: (1) be able to discuss the hypothesized biological elements of psychopathy; (2) have an understanding of the frontotemporal structures and neural networks involved in psychopathy as suggested by genetic, electrophysiological, imaging, and psychopharmacological data; and, (3) be able to discuss the implications of current data for future research and treatment directions.

This presentation will impact the forensic science community by providing a better understanding of how the neurobiology of psychopathy may guide development of effective preventive strategies and treatments. Effective prevention and treatment strategies are of critical importance and interest given the suffering and costs imposed by psychopathic individuals, especially noting that even with increased use of confinement, most such incarcerated persons return to the community.

Psychopathic individuals inflict substantial predatory and impulsive violence. Presently, the principle interventions used to reduce this harm have been confinement and execution. Nevertheless, most psychopathic persons return to the community, giving rise to a need for more effective interventions and treatments. Recent advances in the understanding of the neurobiology of psychopathy hold promise for new research directions and better approaches to treating such individuals. This presentation will review advances in genetics, electrophysiology, imaging, and psychopharmacology relevant to psychopathy with consideration of implications for directions in further research and treatment.

Moreover, the underlying biological elements relevant to developing and sustaining psychopathy will be discussed. Frontal and temporal lobe neural network functions will be reviewed, with attention to specific structures, (e.g., the amygdala, temporal lobe poles, the ventromedial prefrontal cortex, the frontal mirror neuron networks, the fusiform gyrus, and the cingulate gyrus). Additionally, the involvement of temporal lobe and prefrontal cortical signaling, via the uncinate fasciculus, in processing of negative emotional consequences will be discussed. The emergent neurobiological model will then be considered in relation to characteristics derived from factor analysis of the Psychopathy Checklist-Revised (PCL-R), (i.e., interpersonal deficits (impaired affiliative attachments), affective deficits (diminished fear response and impaired empathy), antisocial lifestyle (lack of prosocial goals and deviant behaviors), and antisocial acts (callous disregard for the rights or welfare of others)).

Finally, child-rearing approaches which may promote prosocial development among those harboring the biological substrate for psychopathy will be discussed, as will current psychosocial treatments (e.g., the Risk-Needs-Responsivity model), and preliminary data regarding psychopathic responses to relatively low plasma concentrations of clozapine (glutamatergic novel or atypical antipsychotic). These current data will be considered with respect to directions for future treatment research.

Psychopathy Neurobiology, Psychopathy, Antisocial Personality

I33 Killer Cult Members and the Insanity Plea: Exploring the Line Between Belief and Delusion

Brian J. Holoyda, MD, University of California, Davis Medical School, Dept of Psychiatry & Behavioral Science, 2230 Stockton Boulevard, Sacramento, CA 95817; and William Newman, MD, University of California, Davis Medical School, 2230 Stockton Boulevard, Sacramento, CA 95817*

After attending this presentation, attendees will be able to: (1) summarize the case law regarding cult members who commit murder and later plead not guilty by reason of insanity; (2) describe the role of cult involvement in former and current psychiatric nosology; and, (3) describe practical considerations for forensic examiners tasked with evaluating cult members who commit murder.

This presentation will impact the forensic science community by describing potential changes in the diagnosis of cult members due to changes in the *Diagnostic and Statistical Manual, Fifth Edition* (*DSM-5TM*). Attendees' competence will improve in assessing cult members who have committed murder and then entered a plea of not guilty by reason of insanity.

Cults are charismatic groups defined by members' adherence to a set of beliefs and teachings that differ from mainstream religions. Cult beliefs may appear unusual or bizarre to those outside of the organization, which can make it difficult for an outsider to know whether or not a belief is cult-related or delusional. Some have described cults as a case of mass shared psychotic disorder or as a catalyst for shared psychosis. This raises the important question of whether or not participation in a cult and engaging in behavior that accords with one's cult belief system may be considered psychotic.

The *DSM*, as the primary resource for diagnosing mental health disorders, has provided limited guidance in helping practitioners understand cult involvement and its possible impact on mental health. The diagnostic criteria and supportive text for shared psychotic disorder (or *folie á deux*) in the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision* (*DSM-IV-TRTM*) and its *DSM-5TM* counterpart "delusional symptoms in partner of individual with delusional disorder" within Other Specified Schizophrenia Spectrum and Other Psychotic Disorder do not address cult involvement. Cult participation was mentioned in the *DSM-III* diagnosis of Atypical Dissociative Disorder, in which cult involvement was implicated in causing derealization, depersonalization, and prolonged dissociative states. Though cult involvement was removed from dissociative disorder diagnostic criteria in *DSM-IV* and *DSM-IV-TRTM*, it has returned in *DSM-5TM* criteria under Other Specified Dissociative Disorder as a potential cause of "identity disturbance." Despite this, the degree to which cult involvement may have an impact on mental health functioning remains unclear.

This question becomes particularly germane in a courtroom, as cult members who have committed murder due to their cult beliefs or at the behest of a charismatic leader may attempt to plead Not Guilty by Reason of Insanity (NGRI). It is therefore necessary for forensic experts evaluating cult members to understand how the court has responded to cult members and their beliefs when pleading NGRI for murder. Based on this review of extant case law, cult member defendants have not yet successfully plead NGRI on the basis of cult involvement despite receiving a broad array of psychiatric diagnoses that could quality for such a defense; however, with the reintroduction of cult involvement in the *DSM-5TM* criteria for Other Specified Dissociative Disorder, there may be a resurgence of dissociative-type diagnoses in future cult-related cases, both criminal and civil.

Insanity, Cult, Murder

I34 Civil and Criminal Commitment for Homicidal Ideation

Jason Beaman, DO, Oklahoma State University, 1111 W 17th St, Tulsa, OK 74107; Jennifer Piel, MD, JD*, VA Puget Sound, 1660 South Columbian Way, MS-116-MHC, Seattle, WA 98108; and John P. Shand, MD*, University Hospitals, W.O. Walker Building, 10524 Euclid Avenue, Cleveland, OH 44106*

After attending this presentation, attendees will better understand the role of homicidal thoughts for civil commitment and criminal charges.

This presentation will impact the forensic science community by providing insight into the nature and consequences of homicidal ideation.

Thoughts of killing other individuals are clinically referred to as homicidal ideation. Unfortunately, "thoughts" has multiple meanings and varies among individuals. "Thoughts" may refer to actual thoughts (both welcome and intrusive), fantasies, or threats. It is important to carefully delineate the exact nature of the "thoughts." Homicidal ideation, while shocking, can be a normal human experience; however, there are several studies that suggest that these thoughts have an association with mental illness.[1] Further evaluation has demonstrated that offering individuals with homicidal ideation mental health treatment can be beneficial.[2] In a clinical and forensic setting, the evaluator must carefully evaluate the individual in order to determine what role mental illness and/or substance abuse or intoxication may play. This presentation will discuss the above issues in detail in order to improve the evaluation skills for individuals who encounter persons with homicidal ideation.

The United States Supreme Court held in *Foucha v. Louisiana* that an individual could not be civilly committed for just being dangerous.[3] The opinion of the court was that an individual must be mentally ill and dangerous. Over the last decade, there has been more scrutiny over civil commitment for dangerousness, specifically when an individual discloses (or a provider becomes aware of) thoughts of harming others.

The concept of civil commitment will be discussed. This is a process in which an individual is court ordered to a psychiatric hospitalization for either an evaluation, treatment, or both. The criteria for civil commitment depends on specific legal language and are jurisdiction-dependent. Most jurisdictions in the United States allow for commitment based on "dangerousness to others." In this presentation, the different statutory phrases; specifically focusing on homicidal ideation will be reviewed.

The concept of civil commitment will be applied to an actual clinical case. The nature of the individual's homicidal thoughts and their progression over time will be discussed. The patient's psychiatric symptoms and substance use will be outlined. A review of the clinical Violence Risk Assessment will allow attendees to understand how clinicians gather evidence, then use that evidence to support an opinion while maintaining their ethical obligation to not harm the patient.

Besides civil commitment, an individual may face criminal charges based solely on thoughts and desires of harming other individuals. The actual charge varies based on the severity of the expressed thought. For example, an individual who expresses his thought as a threat could be charged with Terroristic Threatening. Again, this is jurisdiction-dependent. The different forms of crimes that an individual could be charged with for having homicidal thoughts will be discussed. This concept will then be applied in detail to a well-publicized case from New York, in which a police officer was arrested and prosecuted when his thoughts of cannibalizing individuals, including his wife, became known.[4]

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Homicidal Ideation, Civil Commitment, Cannabalism

I35 The Massacre of Erba: An Uncommon Homicide by a "Normal Peaceable" Couple

Federica Collini, MD, Via Mangiagalli 37, Milan 20133, ITALY; and Isabella Merzagora Betsos, PhD, Via Luigi Mangiagalli 37, Milan 20133, ITALY*

After attending this presentation, attendees will be familiar with a famous chronicle case of the Italian press: the massacre of Erba.

This presentation will impact the forensic science community by demonstrating how a common situation of quarreling among neighbors between apparently normal people can turn into a terrible mass murder.

The event occurred at approximately 8:00 p.m. on December 11, 2006, in the small town of Erba, close to Milan, Italy. The massacre took place in an apartment in which four people were killed with knives and iron bars: 30-year-old Raffaella Castagna; her 2-year-old son, Youssef Marzouk; her 60-year-old mother, Paola Galli; and the 55-year-old neighbor, Valeria Cherubini, and her dog. Ms. Cherubini's 65-year-old husband, Mario Frigerio, was present and was saved because he was presumed dead by the killers. After the massacre, the apartment was set on fire.

Raffaella Castagna was attacked and struck repeatedly with a bar (the cause of death was a skull fracture), stabbed twelve times, and then her throat was cut. Paola Galli was stabbed and hit by a bar (the cause of death was a skull fracture). In the apartment located upstairs was the lifeless body of a neighbor, Valeria Cherubini (Mario Frigerio's wife), who had rushed downstairs to help because of the smoke coming from the apartment. She was hit with a pointed, cutting weapon, and seriously injured by 34 stab wounds and 8 blows. Upon arrival of the first responders, the woman was still alive and had cried to the neighbors for help who could not reach her because of the smoke. Ms. Cherubini died by suffocation from carbon monoxide due to the fire, as did the family dog. Mario Frigerio was stabbed in the neck, but survived thanks to a congenital malformation of the carotid artery that prevented him from completely bleeding to death. In the apartment on the first floor, the primary site of the massacre, the small Youssef was assassinated with a single shot to the throat that severed the carotid artery and he died from a hemorrhage.

The investigation first focused on Raffaella's husband, Azouz Marzouk, born in Zaghouan, Tunisia, who had a criminal record for drug dealing and was released from prison on a pardon; however, Marzouk was in Tunisia visiting his parents at the time of the incident. He rushed to Italy, where he was interrogated by the police. Investigators confirmed his alibi and began to investigate two neighbors, Olindo Romano and Rosa Bazzi, because of their suspect behavior and because they had previously had legal disputes with the deceased. These suspicions led investigators to seize some of the clothes of the married couple and to seize their house and car. Both had injuries (the husband had a bruised hand and forearm; his wife a bleeding finger wound). Moreover, the investigators found traces of blood in the car, which was then attributed to Valeria Cherubini. They were stopped by the police the next day. The couple were sentenced to life imprisonment in all three of the Italian proceedings.[1]

This work retraced all the psychological analysis on the two killers, analyzing the acts and the recorded psychological interviews, going over their past lives and traumas, trying to understand the inner reason for their act. The couple was described as very closed and isolated, with a low education level, quiet, anonymous, and morbidly attached to each other. Their relationship was the first for each one; they met each other when they were very young and then married, despite the opposition of his family. Rosa was raped when she was a girl and after the marriage, she lost a baby during pregnancy and could not have more children. This event tied the two pathologically. She was described as a mentally healthy woman, strong and predominant, with an obsessive attention to order and cleanliness, perhaps with an imperceptible mental impairment. Olindo was diagnosed with a clear paranoid trait, deeply in love with and willing to do anything for her. It was their symbiotic relationship that created a new and different entity: the real killer couple.[2-5]

The exceptional aspect of this mass murder lies in the apparent normality of this couple.

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Massacre of Erba, Neighbor Murder, Mass Murder

I36 The Pseudocommando and the Terrorist: Casuistic Comparison and Analysis

Samuel J. Leistedt, MD, PhD, Forensic Mental Health Hospital Les Marronniers, Rue Despars, 94, Tournai, Hainaut 7500, BELGIUM; and Fabienne Glowacz, PhD, Department of Psychology and Criminology, Université of Liège, Quartier Agora, Liège 4000, BELGIUM*

After attending this presentation, attendees will better understand the specific types of mass murderers and the differences between mass murderers and terrorist offenders.

This presentation will impact the forensic science community by providing an in-depth psychological and criminological description of mass murderers and terrorists, including warning signs.

Both pseudocommandos and terrorists kill innocent people. Both generally kill during the daytime, plan their offenses, and generally expect to be killed during the attack.[1-6] In the first part of this presentation, and throughout the study of criminal files, this presentation proposes a causative analysis of two Belgian cases.

On December 13, 2011, at approximately 12:30 p.m. local time, a man identified as NA began lobbing grenades in central Liege, a city in the east of Belgium, not far from the borders of the Netherlands and Germany. The location of the attack is significant. It happened near a judicial courts complex where a seasonal Christmas market had been set up. The area is also a hub for the public bus system. Therefore, it was a bustling, target-rich environment. As a result, four people were killed and dozens more were injured in this attack in the center of Liege.

On May 24, 2014, a single gunman opened fire at the Jewish Museum in Brussels, Belgium. In an attack that only lasted a few seconds, the shooter was able to kill three people and seriously injure another person before escaping. The following week, French police arrested MN during a random search at a bus station in the Mediterranean port city of Marseilles. At the time of his arrest, MN was found to be in possession of an AK-47 rifle and another gun. The AK-47 was reportedly wrapped in a flag bearing the symbol of the Islamic State in Iraq and the Levant. MN was quickly identified as the prime suspect in the Brussels shooting attack. All aspects of the psychopathology and criminology of these offenders will be discussed, including the precise offensive sequences.

The second part of this presentation will compare the psychopathology of mass murderers and terrorist offenders.[1-6] Despite their similarities, these two types of offenders also have clear differences in their psychological and social characteristics.1-6 These differences are very important in terms of motivation, state of mind, and psychopathology. Additionally, these differences are useful for forensic evaluations.

In conclusion, mass murderers and terrorists have common characteristics; however, their basic ways of functioning, psychologically and criminologically, are quite different. These elements are important and should be considered in forensic evaluations.

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Mass Murderer, Terrorism, Psychopathology

I37 Killing a Child: Neuropsychological Profiles of Murderers of Children

Nicole Azores-Gococo, Northwestern University, 710 N Lake Shore Drive, Ste 900, Chicago, IL 60611; Robert Hanlon, PhD, Neuropsychological Associates of Chicago, 645 N Michigan Avenue, Ste 803, Chicago, IL 60611; Saritha Teralandur, MS, DePaul University, 2219 N Kenmore Avenue, Chicago, IL 60614; and Michael Brook, PhD, Northwestern University, 710 N Lake Shore Drive, Chicago, IL 60611*

After attending this presentation, attendees will better understand the heterogeneity of the psychological and neuropsychological functioning of offenders charged with the murder of a child.

This presentation will impact the forensic science community by describing a group of offenders on whom minimal research has been conducted. This study expands the existing research on subgroups of homicide offenders (i.e., filicide and neonaticide offenders) to describe patterns of functioning in a diverse sample, helping to identify psychological and neuropsychological characteristics relevant to forensic evaluation.

An estimated 1,520 children were killed due to child abuse or neglect in 2013 in the United States, accounting for only a portion of all children killed in this country.[1] Despite a disproportionate media focus on women with severe mental illness who murder their children, homicides of children occur in a variety of contexts, not all of which are characterized by mental illness.[2] Studies of offenders who have killed children have been limited to select groups, such as parents committing filicide. Even among these offenders, a variety of motives and patterns of psychopathology are evident.[3-13]

No study has used comprehensive neuropsychological data to compare offenders who kill children. The current study addresses this omission in the literature by examining the demographic, neuropsychological, and psychological characteristics of homicide offenders who have murdered children. Participants were men (n=27) and women (n=6) charged with and/or convicted of murdering one or more children, referred for forensic neuropsychological evaluations. Forensic evaluations included clinical interview, comprehensive neuropsychological assessment, and review of pertinent records. Neurocognitive performance was assessed using standardized neuropsychological tests. Supplementary analyses examined group differences by presence or absence of adult victims, gender, and victim age. P-values were set at 0.05, and significance was two-tailed.

Head trauma (84.8%), psychiatric disorder (57.6%), alcohol abuse (69.7%), and drug abuse (81.8%) were prevalent. Furthermore, a majority (60.6%) had a history of special education. Mean scores were low average in many domains, including overall intellectual functioning (Full Scale Intelligence Quotient (FSIQ)=85.5), attentional functions, immediate and delayed verbal memory, abstract reasoning, executive functioning, inhibition, and verbal skills. Mild to moderate impairments were seen in verbal fluency and processing speed. In supplementary analyses, those who had killed adults as well as children (n=14) had higher scores in many domains, including overall intellectual functioning, executive functioning, verbal memory, and verbal fluency. Differences in background characteristics (e.g., learning disorders) accounted for many of these differences.

This study corroborates and expands upon studies that demonstrated heterogeneous psychological and intellectual functioning among offenders who kill children. Offenders who kill adults in the same offense exhibit higher neuropsychological functioning, are less likely to have a learning disorder, and are more likely to have a personality disorder than those who do not kill adults, perhaps indicating the greater capacity needed to kill an adult and kill multiple victims. Such differences may have implications for criminal responsibility and capacity.

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Neuropsychology, Child Victims, Homicide

I38 Confirmation Bias and Metalinguistic Awareness

Carole E. Chaski, PhD, ALIAS Technology, LLC, Institute for Linguistic Evidence, 25100 Trinity Drive, Georgetown, DE 19947; Elizabeth A. Smith, PhD, University of Quebec at Montreal, CP 8888, Montreal, PQ H3C 3P8, CANADA; Cristina Aggazzotti, MS, Harvard University, Dept of Linguistics, Cambridge, MA 02138; and Ying Liu, BA, University of California-Davis, Dept of Linguistics, Davis, CA 95616*

After attending this presentation, attendees will understand how certain linguistic mechanisms, namely presupposition, epistemic modality, and coherence relations, can inadvertently cause confirmation bias during the forensic science workflow and how metalinguistic awareness can protect the forensic scientist and mitigate bias.

This presentation will impact the forensic science community by providing communicative strategies to mitigate confirmation bias.

Confirmation bias is now accepted as an issue that must be handled proactively to reduce its harmful effects in the forensic workflow from crime scene investigation through adjudication. Confirmation bias is a cognitive predisposition toward a certain interpretation of stimuli or events. Interpretive filters, or biasing mechanisms, are built into human cognition because such filters produce efficiency and speed in decision-making, but cognitive biases can also mislead human cognition: assumptions based on interpretive filters can be wrong, as the well-known Gestahlt switches on visual stimuli (e.g., length of lines, direction of stairs, letter (B)/number (13), and profile/chalice) clearly show. Though the visual examples are more well-known, this presentation focuses on the same effect from auditory stimuli in spoken language. Generally, most cognitive psychologists agree that awareness of the human predisposition to cognitive bias is the first step in mitigating its potentially harmful effects.

But this advice raises two questions regarding implementation: First, how do humans become aware of potentially biasing cognition? Second, how can this awareness outside of the forensic science workflow be transferred into the forensic science workflow in a way that actually may mitigate confirmation bias?

In this presentation, analytical tools from linguistics are presented to demonstrate how metalinguistic awareness — awareness of language not merely as the conveyance of meaning but as an object itself — can be brought to bear on the problem of confirmation bias. In particular, three phenomena within pragmatics are presented: presupposition, epistemic modality, and coherence relations. It is argued that these three areas are especially important for the forensic science workflow. It is also argued that confirmation and cognitive bias can be inadvertently introduced in three steps of this workflow: (1) the interviewing of witnesses/suspects; (2) the presentation of evidence to the forensic science laboratory/examiner; and, (3) the presentation of evidence in court. This presentation focuses on the communication between the crime scene technician and the forensic scientist, but examples of all three steps are provided as constructed examples to illustrate the linguistic pragmatics and as real-case examples to illustrate how the use of presupposition, modality, and coherence relations in workaday communication can inadvertently cause cognitive bias.

A presupposition is information presented as though it is already common knowledge between the speaker and hearer.[1] For example, in (1), the use of "too" presupposes that the hearer carries a gun, which is fine when this has already been established, but dangerous if not, because presuppositions are known to be able to manipulate our memory of events.[2] Examples include: (1) "If I lived around there, I would carry a gun, too."; (2) "We know for sure that this is the boyfriend's blood, we just need the science."; (3) "She then called and threatened to kill him. He was found dead at 6:00 p.m."; and, (4) "He was found dead at 6:00 p.m. She then called and threatened to kill him."

Epistemic modals express the level of certainty with which a person holds a proposition to be true and are marked by words like definitely, certainly, to know that, etc.[3] In examples like (2), the "know" presupposes the conclusion requested from the forensic scientist and inadvertently creates confirmation bias.[4,5]

Coherence relations are relationships between sentences that humans naturally compute to make sense of larger narratives, and the order in which information is presented can affect this computation.[6] For example, in (3) and (4), the same basic sentences are true, but their order leads to different inferences about whether the woman was responsible for the man's death. In short, the examples demonstrate that being aware of the influence of these phenomena helps to avoid their harmful effects. Final suggestions include ways in which a forensic scientist's metalinguistic awareness of these same linguistic strategies can be used to protect the forensic science workflow.

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Confirmation Bias, Pragmatics, Metalinguistic Awareness

I39 Among a German Sample of Forensic Patients: Previous Animal Abuse Mediates Between Psychopathy and Sadistic Actions

Stupperich Alexandra, Police Academy Niedersachsen, Bürgermeister-Stahn Wall, Nienburg (Weser), Niedersachsen 31582, GERMANY; and Micha Strack, University of Goettingen, Georg-Elias-Mueller-Institute of Psychology, Goettingen, GERMANY*

After attending this presentation, attendees will better understand the connection between animal abuse, psychopathy, and sadism. This will be useful for performing risk management and risk analysis on forensic patients.

This presentation will impact the forensic science community by making the link between sadistic actions against humans and animals.

Recent research has uncovered a number of developmental pathways through which humans can develop a tendency to show sadistic behavior during crimes. From a broad field of risk factors, personality traits such as callous, unemotional features (e.g., lack of guilt, lack of empathy, callous use of others for one's own gain) appear early and are relatively stable from childhood to early adolescence.[1] One way of acting out Callous, Unemotional (CU) features is to harm and maltreat animals.[2] In an attempt to explain the relationship of psychopathy and severe violent behavior, this study associates former animal abuse, psychopathy, and sadistic staging within forensic patients. Two topics are addressed: (1) could former animal abuse be identified by a psychopathy checklist profile?; and, (2) does animal abuse statistically mediate between psychopathy and sadistic staging? In a German forensic hospital, 60 patients were investigated. Animal abuse was assessed via Face-to-Face (FtF) interview, Psychopathy Checklist:Screening Version (PCL:SV) was administered, and sadistic staging was identified by file records.

Discriminant analysis separated former animal abuse by high adolescent antisocial behavior, superficialness, lack of remorse, lack of empathy, and grandiosity. Discriminant analyses separated the 10 animal abusers from the 50 non-abusers by a weighted function of the 12 PCL:SV items ($Chi^2_{(12)}$=36.44, p <.001; 87% correct classifications, *kappa*=.61). The discriminant function could be interpreted by its loadings of high adolescent antisocial behavior (*r*=.63), superficiality (*r*=.50), lack of remorse (*r*=.45), lack of empathy (*r*=.40), and grandiosity (*r*=.39). Animal abusers scored significantly higher than non-abusers.

These findings fit with Porter, Campbell, Woodworth, and Birt, who found that psychopaths who killed showed higher factor 1 scores.[3] They concluded that the psychopath's aggressive behavior is controlled, not affective. Using animals as early targets may be an expression of this controlled predatory behavior: on the one hand, animal abuse necessarily implies controlling a creature, but on the other hand, the psychopath learns how to control his own feelings and actions. Additionally, the obsequious behavior of animals may increase the psychopaths' superficial and grandiose feelings.

The second goal of this study was to analyze the pathway among psychopathy, animal abuse, and sadistic acting in forensic patients. The mediation from psychopathy to sadistic staging through animal abuse was found to be a complete one. Sadism and psychopathy have often been theoretically and clinically associated.[4] By prediction of sadistic acting using the total psychopathy scores, the findings were replicated and analyses of animal abuse foster that association. The results, although limited in sample size, fit with a model of animal abuse as a causal step toward sadistic crimes. Hence, information on animal abuse supports risk analysis.

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Psychopathy, Sadism, Animal Abuse

I40 Resolving Ethical Dilemmas Using Dialectical Principlism in End-of-Life Decisions

Robert Weinstock, MD, 1823 Sawtelle Boulevard, Los Angeles, CA 90025; and William C. Darby, MD*, UCLA, 760 Westwood Plaza, C8-193, Los Angeles, CA 90024*

After attending this presentation, attendees will acquire methods of analysis to help resolve ethical dilemmas.

This presentation will impact the forensic science community by analyzing conflicting considerations in end-of-life decisions.

Forensic scientists encounter serious ethical dilemmas when they face conflicting obligations with no one solution satisfying all of their concerns. It can be a challenge to determine the best action in such complex situations without a systematic approach. As a result, it is helpful in difficult situations to lay out the issues, prioritize, and balance the potentially conflicting duties to determine what is most ethical. The method of doing this was developed by Weinstock and Darby and is called dialectical principlism. "Dialectical" dates back to ancient Greece and refers to the process by which apparently contradictory competing considerations are synthesized into a coherent whole. "Principlism" refers to all types of relevant principles such as biomedical ethical principles: autonomy, beneficence, non-maleficence, justice, personal ethical values, and protecting the vulnerable in our society, among others.

Forensic scientists must consider in a specific context which duties are primary and which are secondary in a particular role. As a physician, the primary duty revolves around the patient's welfare. Usually, primary duties will outweigh secondary duties and considerations of all types. But there can also be conflicting primary duties. They should be balanced in a way to impinge as little as possible on each other. In difficult end-of-life conundrums, the dilemmas are usually caused by conflicting primary duties. In this case, the relevant primary duties are beneficence, non-maleficence, and autonomy. Dialectical principlism can help practitioners determine their most ethical action.

Physician-assisted suicides are now legal in certain states and raise important ethical considerations on how these laws will affect end-of-life decisions. Physicians must have a high threshold to rule out psychiatric illness, cognitive impairment, and false expectations of prognosis that may impede on a person's ability to choose for assistance in suicide. These new laws may have the unintended effect of increasing patient suicide in situations in which treating the underlying illness, providing further medical education and counseling, or allowing time itself to impact a person's decision-making. This is not to say that in certain circumstances, physician-assisted suicides may be an ethical means to reduce human suffering. Examples are cases where there is a highly unfavorable prognosis, for an irreversible and untreatable disease process associated with significant physical or emotional pain or suffering, and with the patient having a clear understanding of their likely prognosis and without their judgement being clouded by psychiatric or cognitive problems.

The dialectical principlism framework can be used to demonstrate how the physician's role and subsequent duties and considerations are balanced to determine the most ethical action in these types of end-of-life dilemmas. Current law should be changed and future laws made to include stronger safeguards to be sure that people wanting physician-assisted suicide fully appreciate the nature of their situation when they make these decisions.

Ethics, Dilemma, End-of-Life

I41 Effects of Anger Management and Social Support to Cope With Cyber Bullying of Adolescents

Nursen Turan, MD, Marmara University, Medical Faculty, Forensic Medicine Dept, Basibuyuk, Maltepe, Istanbul, TURKEY; Asligul Metin, Marmara University Medical Faculty, Basibuyuk Kampusu Maltepe, Istanbul, TURKEY; Sazimet Geyik, Marmara University Medical Faculty, Basibuyul Maltepe, Istanbul, TURKEY; Sitti Hatice Nur Nas, Marmara University Medical Faculty, Basibuyuk Kampusu Maltepe, Istanbul, TURKEY; Berfin Aydogdu, Marmara University Medical Faculty, Basibuyuk Kampusu Maltepe, Istanbul, TURKEY; Burcu Kilic, Marmara University Medical Faculty, Basibuyuk Kampusu Maltepe, Istanbul, TURKEY; and Yesim Yenigul, PhD, Pendik Hospital, Pendik, Istanbul, TURKEY*

WITHDRAWN

I42 Juvenile Sex Trafficking

Sara R. Thomas, MS, Georgia Bureau of Investigation, 3121 Panthersville Road, Decatur, GA 30034*

After attending this presentation, attendees will better understand sex trafficking within the United States as well as the psychological and physical consequences of sex trafficking on victims.

This presentation will impact the forensic science community by providing education and awareness of juvenile sex trafficking.

Sex trafficking — a form of human trafficking — is a widespread problem, both globally and within the United States. It is considered to be the most prevalent form of modern-day slavery and trafficking in persons has been identified as the second-largest criminal industry in the world, only behind illicit narcotics trafficking.[1,2] Sex trafficking touches numerous disciplines including criminal justice, medicine, education, and social services. It further affects communities and individuals of all ages, genders, races, socioeconomic statuses, and religions.

While the full extent and consequences of the sex trafficking problem is still largely unrecognized, current research statistics estimate 15,000 to 50,000 annual cases of sex trafficking each year.[3] Of those victims, the majority are women and children; however, exact statistics related to the prevalence of sex trafficking are difficult to obtain due to the unique characteristics of victims and the difficulty in identifying victims.[4,5]

Trafficking victims are unique, demanding specialized investigative techniques and victim services. This presentation will discuss general forms of force, fraud, and coercion used by offenders, as well as the tactics and techniques used to lure victims into the sex trade and the characteristics of sex trafficking victims and offenders. This presentation will address the physical and psychological stressors faced by trafficking victims, the long-term consequence on the victims, and the barriers faced while working with victims of sex trafficking, such as the psychology of victimization and trauma bonding, venues of trafficking within the United States, and its correlation to runaway and missing youths. This presentation will include suggestions for future research and resources available for personnel that work with trafficking victims.

In addition to a primer on general law enforcement response to juvenile sex trafficking, this presentation will include a case study of the criminal investigation and prosecution of Darryl Curry ("DC Pimp") who was arrested and convicted for juvenile sex trafficking. The case study will discuss the physical and psychological control techniques used by Curry to force his victims to engage in the sex trade as well as utilize victim statements to illustrate the psychological impact on the victims. This presentation will additionally use photographs of physical injuries to victims and videos made by Curry instructing others as to how to exploit victims, and will discuss the use of Special Weapons And Tactics (SWAT) personnel during a barricaded incident.

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Sex Trafficking, Juvenile, Victim Psychology

I43 Empirical Survey in the Italian Courts

Laura Volpini, PhD, via dei Sulpici, 62, Rome 00174, ITALY; Roberta Russo, MS, Via Sottoporta, Castelmola, ITALY; Federica Rossi Berluti, MS, Via leone Tolstoj, L'Aquila, Abruzzo 67100, ITALY; and Cristina Mazza, Str. Mammagialla 3B, Viterbo, ITALY*

The goal of this presentation is to define the standards and good procedures in order to consider the opinion of the expert valid and reliable on the basis of empirical evidence.

This presentation will impact the forensic science community by minimizing the discretionary power of each expert and providing all the people involved in the juridical context with professional standards.

The case of child custody litigation is governed by the Italian Law 54/2006 concerning *joint custody*, according to which the minor has the right to maintain an impartial and continuative relationship with each parent, to receive care, upbringing, and education by both parents, and to maintain a significant relationship with ascendants and relatives of both parental branches. In case of litigation, considering that parents are unable to agree on the custody of the children, the judge may have recourse to have collaboration of an Expert Witness (EW); an expert able to evaluate parenting skills and risk and protection elements concerning the entire family unit. The EW, by means of an explicative report written on the basis of clinical interviews and instruments of psychodiagnostic assessment, provides an opinion on parenting skills and on the appropriate method of custody; however, shared standards and good procedures currently need to be defined in order to consider the opinion of the expert valid and reliable, on the basis of empirical evidence.

The present qualitative-quantitative exploratory research begins from the previously mentioned statements. The sample, which consisted of 116 files established at the first instance by the courts in Velletri, Italy, and Viterbo, Italy, was randomly chosen and refers to the period of 2003-2014. The files were chosen from documents which met the following requirements: legal proceedings of judicial separation of a husband and wife concerning a minor's custody and an EW was required to evaluate parenting skills beginning from 2006 (in other words, after the Law 54/2006 concerning joint custody came into being). The objective was to examine the method used by the EWs and to investigate the criteria adopted by the judge in the final decision.

A survey was performed by using an ad hoc analysis form divided into specific macro-areas: (1) personal data; (2) legal proceedings; (3) method adopted by the EW; and, (4) the final decision of the judge. The analysis of the data regarding the method adopted by the EWs reveals that most mechanisms were used to evaluate the personality of both parents and minors. In this regard, relational dynamics in the family unit lost their importance and therefore the EW method strayed from the primary field of research, specifically parenting skills. The assessment was supported by audio/video recording only in a few cases. The two most-used scientific criteria applied to evaluate parenting skills were the access criterion (30%) and the criterion of the psychological parent (29%). In 63% of the EW cases, according to Law 54/2006, joint custody was considered the best solution. In most cases, the final decision of the judge is in line with the conclusions drawn by the designated expert. Since the opinion of the EW plays a large role in the formulation of the final decision, it is clear that unambiguous guidelines are needed to minimize the discretionary power of each expert and to provide everyone involved in the juridical context with professional standards. The final goal is, therefore, the possibility of playing each role in a responsible manner, both professionally and morally, in order to guarantee the execution of the parental role in the exclusive interest of the minor.

Child Custody, Litigation, Parental Skill

I44 I Need to Be Myself, I Can't Be Anyone Else — Analyzing the Role of Forensic Sciences in Disorders of Sex Development (DSD), Discussion of Historical Case Studies, and Contemporary Reports Leading to New Perspectives

Annarita Franza, PhD, Via Delle Oche 15, Florence, ITALY; and Vincenzo Lusa, JD*, Via Ferdinando, Palasciano #72, Rome 00151, ITALY*

After attending this presentation, attendees will understand the role of the forensic sciences in the investigation of sex and gender issues in individuals with DSD (i.e., congenital conditions in which development of chromosomal, gonadal, or anatomical sex is atypical).

This presentation will impact the forensic science community by presenting a multidisciplinary approach based on an analysis of historical case studies and contemporary reports that will demonstrate how forensic science can assist in identifying the biological sex, gender identity, and social-sex role in a person with a sex or gender non-conformity.

The birth of individuals that are neither male nor female has always fascinated scientists and several cases of gender ambiguity have been documented throughout history, including the case of Christian Wasa, better known as Sweden's Queen Christina. On December 8, 1626, King Gustav II Adolf and his wife, Queen Maria Eleonora, celebrated Prince Christian's birth. One week later, the newborn was recognized as female. Nevertheless, she was proclaimed king, not queen, by the Swedish parliament in 1632. On the occasion of the 390th anniversary of her birth, the hypothesis of DSD will be examined in light of such rare documents relative to Queen Christina's medical biography such as her private physicians' evaluations, an anonymous clinical account kept in the Vatican Library describing her last illness, and the autopsy record now in the Austrian State Archives. Queen Christina's physical and personal traits will then be compared to her portraits as well as to the data collected during her exhumation in 1965.

One of the first scholars to focus on crime and gender diversity was Cesare Lombroso (1835-1909). In *Love in Insane People* (1881), he saw the ambiguity of sex differentiation as a "degeneration" that caused certain people to commit crimes. The case studies listed in *Crimes of the Libido* (1886) will be analyzed jointly with the forgotten *A Strange Case of Cross-Hermaphroditism in a Female Maniac* (1867) in which Lombroso correlated Maria F.'s ambiguous genitalia, criminal behavior, and mental health to congenital brain anomalies that he similarly observed in her twin sister. Lombroso stated how a person with sexual identity disorder has "the right to be treated differently" by the medicolegal system. Each case should have been evaluated by a panel of experts in pathology and jurisprudence in order to assign a sex, if needed, without rash decisions being made — a procedure similar to the Multidisciplinary Teams (MDT) present in centers for DSD management.

Lombroso's essay was mentioned by Cesare Taruffi (1821-1902), professor of pathology at University of Bologna, in *Hermaphroditism and Teratology* (1902) relative to Johann Casper's (1796-1864) works on the *Prussian Criminal Code* (1856-1864). Taruffi discussed gender assignment in infants with ambiguous external genitalia, recommending sex reassignment after 18 years of age in the presence of a medicolegal expert who would certify the conformity of the patient's anatomical and psychological sex in keeping with his/her status and gender roll. Taruffi's analysis underlined the issue of legally recognizing a "third gender," a process started in 2013 when Germany allowed an "indeterminate" gender option on birth certificates. In 2014, both Australia and India permitted the registration of a person's sex as "non-specific."

Nonetheless, individuals with DSD still face disadvantages throughout the legal system. In 2000, Miki Ann DiMarco spent 438 days in the Wyoming Department of Corrections' most restrictive and isolated housing pod due to the fact that she was "classified as an individual of ambiguous gender," demonstrating the difficulty in determining appropriate housing arrangements in the prison system for people with DSD. In 2014, the Bureau of Justice Statistics indicated that both adult and juvenile offenders reported higher rates of sexual victimization while in custody. Also, what happens when he/she is the victim of a crime? Currently, crime-reporting variations due to DSD are under-researched in victimology, although a survey conducted in 2013 using the Theory of Planned Behavior (TBC) showed that people with DSD are less willing to report crimes out of fear gender-based discrimination.

In conclusion, DSDs present a unique challenge in terms of medicolegal management; increasing the focus on forensic sciences will help to protect a person's sexual identity beyond stereotypes and prejudices.

Disorders of Sex Development, Sexual Identity, Crime Reporting

I45 Hypnosis in the Court Room

Sebastien S. Prat, MD, St Joseph's Centre for Mountain Health Services, Forensic Psychiatry Dept, 100 W 5th Street, Hamilton, ON L8N 3K7, CANADA; and Joseph Ferencz, MD, PhD*, St Joseph's Healthcare, Forensic Psychiatry Program, 100 W 5th Street, Hamilton, ON L8P 3K7, CANADA*

After attending this presentation, attendees will be aware of the application of hypnosis in the court room and its scientific and legal status.

This presentation will impact the forensic science community by providing legal and clinical information about this controversial technique used to bring evidence during a trial.

Hypnosis is a technique which has been practiced by mental health professionals for more than a century. It has been used in the diagnosis and treatment of a variety of psychiatric disorders, particularly those with an element of unconscious behavior or "automatism" (e.g., Dissociative Identity Disorder). Hypnosis is considered an induced state of consciousness requiring a degree of suggestibility and has been used in the courts to obtain evidence related to states of "automaticity" as well as to "recover" previously unavailable memories. Another controversial aspect of hypnosis is its implication in crimes in which the accused declares that his behavior was induced by hypnosis and therefore he did not have any control over it, raising a potential non-criminally responsible defense. This aspect continues to be debated, with some experts arguing that the criminal behavior happened in an "automatic" state, whereas other experts consider there is insufficient scientific evidence to support that hypnosis sufficiently alters the mind of an individual to thereby deprive him of his conscious volition. A similar issue arises in the case of Dissociative Identity Disorder, the theoretical basis of which is similar to hypnosis, although one is considered as a therapeutic technique and the other as a disease of the mind. The concept of dissociation, originally developed by Pierre Janet in the 19th century, will be examined with particular regard to its relationship to hypnosis.

The role of hypnosis in the courts has been a source of much controversy and, in recent years, many jurisdictions have come to exclude such evidence. For example, in 2007, the Supreme Court of Canada decided to no longer recognize hypnotically based evidence in the courtroom. This presentation will review many of the key Canadian and United States cases which have led to the current view of hypnosis in the courts.

Trial, Hypnosis, Evidence

QUESTIONED DOCUMENTS

J1 Evaluation of the Problems in the Field of Questioned Documents in Turkey

Isil Ocal, Cukurova University, Faculty of Medicine, Biophysics Dept, Adana 01330, TURKEY; and Mete K. Gulmen, PhD, MD, Cukurova University, School of Medicine, Dept of Forensic Medicine, Adana 01330, TURKEY*

After attending this presentation, attendees will gain an understanding of the problems and suggested solutions regarding forensic document examination and expertise in Turkey.

This presentation will impact the forensic science community by presenting the idea that there are serious system problems in forensic document examination in various parts of the world and by making attendees aware of Turkey's current international crime status.

In Turkey, two separate law enforcement agencies conduct forensic examinations of questioned documents in crime investigations on behalf of prosecutors. The Turkish National Police (TNP) and the Gendarmerie Criminal Forensic Department (JKDB) are assigned jurisdiction over crime investigations based on the regional location of the crime's commission. In urban areas, all forensic criminal investigations are managed by the TNP; in rural areas and districts which do not have police organizations, criminal investigations are within the jurisdiction of the JKDB. All laboratories are located in major cities and serve the provinces they are located in as well as the surrounding provinces. Forensic examinations include forensic questioned document services.

The TNP and JKDB are not planning to increase the number of special forensic laboratories but have proposed to replace existing laboratories by building laboratories large enough to meet the increasing demand. Current laboratories are too small to house the number of personnel conducting examinations and, more importantly in the future, the current facilities lack the specifications of modern forensic facilities. Laboratories were established by both organizations with special focus on geographical location, workload, and need.

The demand for forensic document examination has intensified over the years due to an increase in crime along with population, introduction of new laws during the European Union (EU) acquisition process, and new forensic analysis techniques. Today, the primary problem facing questioned documents in the forensic sciences of Turkey is the lack of qualified academic and laboratory staff. Most practitioners who are qualified have completed minimal formal education requirements and lack foundational knowledge in forensic sciences. The judiciary and all levels of the legal system are encountering serious problems and deficits in Turkey. This presentation will discuss the qualification methods for legal practitioners and the judiciary in Turkey as well as the main problems in the current expertise of this system.

The police organization can be judged as a relatively efficient organization. However, the methods of forensic document examination have to be aligned with European standards as far as forensic science is concerned. It was through this process that the need for forensic document examination services in Turkey became apparent.

The expert problems encountered in questioned documents in the forensic sciences field in Turkey and the solutions offered will be discussed and evaluated in detail.

Questioned Documents, Forensic Laboratories, Turkish Judicial System

J2 Electrostatic Detection Apparatus (ESDA®) for Questioned Document Examination — Theory and Application

F.L. Jim Lee, Jr., MS, Summit Forensic Document Examination Lab, PO Box 207, Eden, UT 84310*

After attending this presentation, attendees will have an understanding of the theory and application of the ESDA®, an instrument designed, manufactured, and sold by Foster + Freeman Ltd.

This presentation will impact the forensic science community by equipping attendees with the theory and practical application knowledge in the area of questioned document examination and in the development of indented writings and entries using the ESDA®.

ESDA® is a specialized instrument, generically referred to as an Electrostatic Detection Device (EDD) that is designed for the development and visualization of indentations in the surface of paper that may transfer when a single sheet of paper is placed on top of a stack of several sheets of paper and then written upon. The technique is acute enough in its sensitivity that it is capable of detecting indentations on sheets of paper that are several layers below the top sheet of paper. Additionally, similar to latent fingerprint development, by using this apparatus and technique, indentations may be revealed that were created many years before conducting the examination using the ESDA®.

The ESDA® provides the document examiner with a unique facility for detecting the surface irregularities associated with "indented" or "impressed" writing. While traditional methods and techniques involving illumination by various angles and intensities of light are limited to the detection of simple surface indentations, the ESDA® process, while a bit complex but fairly straightforward in application, will be described in detail during this presentation, showing why and how it responds to more subtle surface abrasions caused by paper-to-paper movement that may occur during the act of writing.

This presentation will cover an overview of the ESDA® process, a description of the resulting image from the process, and controllable factors that may impact results such as document moisture content, ambient humidity, and paper type. Additionally, this presentation will discuss the theory behind and the application of the ESDA® in revealing indented writings and impressions in paper. Also to be discussed are the various methods of toner application in the process, such as the Cascade method, the Aerosol Spray method, the Toner Application Device (TAD) method, and the Brush method used with some other models of EDDs. Additionally, the evaluation of EDD results, both during the pre-examination phase and post-examination phase, will also be discussed. Further, the preservation of the results from an examination using an EDD will be addressed.

While the ESDA® is primarily used for the examination of questioned documents to visualize and decipher indented writing that may be present on the document, the instrument and the technique can reveal the presence of fresh fingerprints and paper fiber disturbance of the surface of paper, potentially indicative of mechanical erasure or the detection of footwear impressions on paper.

ESDA®, Indented Writing, EDD

J3 A Study of Bandings in Printed Black Texts for the Identification of Monochromic Laser Printers

Ning Liu, MA, 87 Bailongjiang East Street, Apt 10-3-901, Nanjing 210019, CHINA*

After attending this presentation, attendees will understand: (1) the basis of the banding frequencies presented in printed black texts which can be the individual features of laser printers; and, (2) the improved methods of extracting the banding signals from printed black texts.

This presentation will impact the forensic science community by providing a way to use banding features in printed black texts as an individual feature for the identification of monochromic laser printers.

Banding artifacts caused by photosensitive drum velocity variation or its resultant scanline spacing variation were often perceived in outputs of monochromic laser printers as periodic light and dark bands perpendicular to the print direction in halftone images as well as in texts. This presentation includes three parts. First, this presentation addresses the fact that a set of specific banding frequency components characterized the class signature of a laser printer while the banding signals with the highest intensity often exhibited in the printed black texts. This corresponded with its primary frequency components shown in its frequency spectrum analyzed with fast Fourier transform. Other than the gear transmission errors that have been proven to be the sources of the output density fluctuations in various research, there were some primary banding frequency components that occurred from unknown sources. By detecting the angular velocity variation of photosensitive drums with an attached gyro sensor, experiments were conducted to locate the sources of the undetermined frequency components of the two models of laser printers: HP® LJ 1020 and HP® LJ P1008. Validation of both data was established by comparing the banding signals extracted from outputs to the signals detected with the gyro sensor.

Next, the performance of banding signals in printed black texts was investigated. Equipped with the original equipment manufacturer cartridges, 200 devices of the two models of Hewlett-Packard® laser printers were sampled and analyzed. The primary banding frequency components, as their individualities, were often presented in black texts printed by the tested printers. Third, the results of the banding extraction methods were compared. The bandings within printed black texts larger than 36pt were extracted with three methods: scanning in film mode, reflectance transformation imaging, and laser scanning confocal microscopy. The extracted signals from the digital images obtained with these three methods were compared to determine a method of improving the signal-noise ratio of banding frequency.

This study shows that banding artifact can be a promising feature for discriminating documents printed by individual laser printers of the same type, even for text-only documents, if the banding signals in black texts are detected.

Laser Printer Identification, Banding Frequency, Black Text

J4 Determination of the Sequence of Non-Intersecting Lines From Laser Toner Particles and Pen Ink by Stereomicroscope

Ismail Çakir, PhD, Institute of Forensic Sciences, Istanbul Universitesi, Adli Tip Enstitusu, Cerrahpasa Kampusu 34098 Fatih, Istanbul , TURKEY; Gürsel Çetin, MD*, Istanbul Üniversitesi, Cerrahpasa Tip Fakültesi Cerrahpasa Fatih, Istanbul 34098, TURKEY; Abdi Ozaslan, MD, Istanbul University, Cerrahpasa Medical Faculty, Forensic Medicine Dept, Fatih, Istanbul 34098, TURKEY; and Ibrahim E. Çaki, Istanbul University, Cerrahpasa Tip Fakültesi Adli Tip Anabilim Dali, Istanbul, TURKEY*

After attending this presentation, attendees will better understand the Leica® EZ4 stereomicroscope and its use in determining the sequence of non-intersecting lines.

This presentation will impact the forensic science community by discussing the Leica® EZ4 stereomicroscope's use as a magnification tool for the determination in the sequence of non-intersecting lines.

In cases involving a document signed for one purpose that has then been turned into a promissory note, determination of the sequence of the intersecting lines contributes significantly to the analysis of the case. In the absence of such a situation, determination of whether the ink, toner, and/or mechanical parts of a printer or photocopy device used in creating promissory notes overlap the signature provides significant information in the fraud investigation process. In this study, a case using the Leica® EZ4 stereomicroscope and the results of determining the sequence of non-intersecting lines will be discussed.

Case: An original promissory note in the amount of $500,000 was issued on January 14, 2009, by the creditor M.Ü. to the debtor E.H.İ. with a payment date of August 15, 2013. A second original promissory note with an issue date of January 14, 2009, by the creditor M.Ü. to the debtor E.H.İ. with a payment date of September 15, 2013 was also submitted for examination. Each promissory note contained the signature of the creditor and both signatures were examined.

In the physical examination of the notes, it was determined they were not press-printed and the lower and upper edges of the bills were not form-cut. For the note with the payment date of August 15, 2013, the horizontal width was 21cm (A4 size), the left-side height was 9.6cm, and the right-side height was 9.4cm. The horizontal width of the note dated September 9, 2013, was 21cm (A4 size), the left-side height was 10.1cm, and the right-side height was 10.2cm. With notes that are standard size and are not press-printed, fraud can be committed by using a signature placed on a blank sheet in three locations: before printing the promissory note text, between the text on the upper part, and between the text on the lower part.

Examination of the two notes using the Leica® EZ4 stereomicroscope and the S520® Document Detector CTMS® document examination device, print traces and light ink reflections and dispersions on the signatures of the documents were observed. Based on this examination, it was determined that the signatures and names were created with pen. There was no evidence of physical and chemical deletions on the documents. In the examinations performed, toner dispersions and traces were left by the laser writer on the printed letters and line frames on the documents. It was detected that there were partial losses in the letter-number characters due to the device and toner used (some parts of letters and numbers were not printed) and there was an intense heterogenic toner particle distribution which covered the entire front side of the document. Toner particles were observed intensely on the entire surface of the document in the areas where printing traces of the signature was not present, but were not observed in the large parts where internal deep printing traces of the signature were located. In the examination performed with stereomicroscope, the debtor signatures on the notes were written in ink on the paper first and the promissory note text was printed afterward above these signatures through a computer and laser writer.

Considering the width of the paper, a blank A4 paper or a paper with a letterhead and/or text was signed, then the promissory note text was printed via a computer and a laser writer, followed by the present notes being created by cutting the upper and lower parts of the paper.

The most distinctive finding in this instance in terms of fraud is the toner particles over the signature lines and the absence of these particles under the ink which demonstrates that the note was printed after the signature. The fact that the promissory note was cut not only from one side but from both the upper and lower sides of the paper is the second finding for this determination.

Sequence of Writing/Printing, Toner Particles, Ballpoint Pen Ink

J5 Oh Brother — Another Paper on Following the Basics

Thomas W. Vastrick, BS, 522 S Hunt Club Boulevard, Ste 217, Apopka, FL 32803*

After attending this presentation, attendees will have a fresh new reason to follow published methodology standards and not take shortcuts.

This presentation will impact the forensic science community by demonstrating why there are published standard methodologies and why they work.

This study involes a case of a Last Will and Testament where an attorney provided a copy of the will and more than 400 known specimens. Pretty basic, one might think and, in a manner of speaking, it was. Yet the path was strewn with landmines ready for any unsuspecting shortcut-taker. Was the use of a copy adequate for comparison? The document was a good quality copy and there is research to show that forensic document examiners can properly assess the features of genuineness and non-genuineness on good quality copies up to a 96% confidence level. The original will was on file in the courthouse. It seems a simple matter to just go to the courthouse to view the original, yes? No. The original was in a courthouse in Bangalore, India.

The known specimens had the three "C's" for being proper comparison material — comprehensive, comparable, and contemporaneous. Pretty basic, one might think. Pretty good specimens, one might think. An examiner should be able to compare these signatures with the questioned signatures without any complications. The signatures were of a complex nature and appeared to be fluently written, meaning no discernible poor line quality and plenty of evidence of speed of execution. Feature-by-feature one could find almost every characteristic noted in the questioned signatures somewhere in the known specimens.

Yet three examiners testified. One examiner testified that the signature was genuine and two testified, "NOT SO FAST."

The deceased was a former member of the Indian Parliament who had property holdings in India, England, and the United States worth tens of millions of dollars. The contested Last Will and Testament left his entire estate to his brother and business partner. Arguing against the Last Will and Testament was the deceased's daughter. In a male-oriented society, the directive of the Last Will and Testament was not considered unusual. The United States case was the first to go to trial. Foreign jurisdictions had no obligations to recognize the decision of the United States court verdict but it was generally acknowledged that the party on the losing end of this decision was going to have a very difficult time obtaining a contradictory verdict in either India or England.

The trial was held in Harris County in Houston, TX, before a jury of six. During the course of the trial, family from India traveled to Texas, two document examiners traveled from other regions of the United States to testify, one document examiner traveled from overseas to testify, and one witness became seriously ill on the witness stand, delaying the trial by a week. In the end, the jury verdict was six to zero in favor of….

This presentation will walk you through the examination and the extraordinary steps that were required just to follow the basics, and explain how sticking to published standard methodologies provided the guidance needed to find the truth and protect the true heir of a substantial estate. This case has the makings of an exciting book — and it all really happened.

So is it a complex case or just an ordinary everyday examination? The audience can decide.

Signature, Forgery, Handwriting

J6 Counterfeit Detection Training in Distributed Learning Environments

Joel A. Zlotnick, MSFS, U.S. Department of State, 600 19th Street, NW, Ste 12.601, Washington, DC 20522; Zhengfan E. Song, MS, Diplomatic Security Training Center, 2220 Gallows Road, Dunn Loring, VA 22027; and Tyra Lundy, MS, All Native Group, 2230 Gallows Road, Ste 300, Dunn Loring, VA 22027*

After attending this presentation, attendees will gain an understanding of factors to consider when planning counterfeit document detection training programs for large and geographically diverse audiences.

This presentation will impact the forensic science community by providing a framework for deploying counterfeit document detection training using a combination of distributed learning, classroom training, and hands-on exercises.

In every discipline of forensic science, the most comprehensive examinations of evidence are completed by individuals with substantial education, training, and appropriate laboratory facilities. Logistical and resource considerations mandate efforts to push analysis into field environments so that preliminary information can be obtained quickly by personnel with less training and fewer resources. Examples include field drug-screening tools or presumptive tests for blood. In questioned documents, fraud detection is frequently the task of individuals for whom it is not a primary job duty (examples include bank tellers, police officers, and motor-vehicle employees). Training is critical to prepare these individuals for preliminary field counterfeit detection.

To design organizational counterfeit detection training for a large population of non-experts, several questions must be answered. Are the training recipients a large or small audience and where are they located? Will the training cover detection of a limited set of documents or a wide variety of documents? Are genuine, counterfeit, or altered documents available for hands-on exercises in or outside of a classroom environment and in what quantities? How can the behavior of complex security features that defy photography be displayed on a monitor? The answers provide important needs assessments about the document types and the balance of e-learning to classroom training.

In the United States Department of State, the Bureaus of Consular Affairs and Diplomatic Security have developed a broad strategy for deployment of fraud document detection training to a geographically diverse mix of consular and law enforcement staff that must assess a broad range of national identity and travel documents. While training on individual country documents is critically important at the local level, it is not possible for headquarters offices to build local document training for every location. Therefore, the training strategy is feature-based instead of document-based and provides trainees with an understanding of similar document security features as they are used across document types. For example, training on watermarks demonstrates watermark features in several foreign passports, birth records, and currencies to illustrate commonalities across documents of different types and from different issuers. Then, when presented with an unfamiliar document, the trainee can readily self-train on and evaluate a new watermark or other security features.

The Department of State possesses adequate quantities of genuine documents it issues for these to be used for hands-on exercises at all posts, but foreign and counterfeit documents are more restricted. Images of features in foreign documents are deployed in an e-learning format to reach the largest audience possible, with liberal use of alternate light images, video, and animation. Trainees are asked to locate the same technology in a set of physical United States documents as a hands-on exercise. By simulating different tools, angles, and lighting conditions, this distributed learning program allows trainees to examine, touch, and tilt the documents virtually, with a similar training experience that they can get in the classroom-based programs. It also serves as a job aid and provides the consular and law enforcement staff in the field with a continuity of on-demand training without having to be constrained by schedules and travel costs. For counterfeit document training, classroom exercises are necessary for two reasons: (1) because of the difficulty in demonstrating certain modalities of fraud on screen and in the absence of physical counterfeits; and, (2) because counterfeit or altered documents are in restricted supply compared to genuine documents.

This interactive distributed-learning model can be applied to many security training areas where learners need hands-on experience, continuous and exact feedback, and the opportunity to use the tools at any time. For example, the Diplomatic Security Training Center has developed training simulations of door locking systems, X-ray scanner training, hydraulic vehicle barrier systems, and simulated use of criminal databases. For both initial learning and for quick refresher training, well-designed training simulations are both very effective and very efficient.

Counterfeit, Training, E-Learning

J7 A Survey of Usage of Opinion Terminology in Questioned Document Examination and on Varying Proposed Approaches to the Standardized Terminology

Carl R. McClary, BA, 2600 Century Parkway, Ste 410, Atlanta, GA 30345*

After attending this presentation, attendees will understand what types and levels of opinion terminology are used by practitioners in the questioned document discipline. Alternative reporting language will be explored and comments on each will be given. This presentation will also afford the attendee the mission of the Organization of Scientific Area Committees (OSAC) task group on opinion reporting and the action plan for this group going forward.

This presentation will impact the forensic science community by providing an insight as to what language, exactly, is being utilized in the discipline among both government and private examiners. Alternative language being considered will be offered and opinions on the same.

Since the publication of Thomas McAlexander's "The Standardization of Handwriting Opinion Terminology" in the *Journal of Forensic Sciences* in March 1991, there has not been widespread usage of any other compendium of opinion terminology in the questioned document discipline in either the government or private consulting sectors. The 1996 publication *E1658 Standard Terminology for Expressing Conclusions of Forensic Document Examiners* by the American Society for Testing and Materials (ASTM) provides the basis of Mr. McAlexander's recommendation, with few edits published since the current 2008 version. The 1991 *Journal of Forensic Sciences* piece cites the case for usage of probability statements by document examiners to reflect gradations of certainty. Mr. Alexander explained that probability "is not a statistical measurement but a measurement of the examiner's confidence, based on scientific principles and experienced judgement, that the opinion rendered is correct. This is true because probability relates to qualitative as well as quantitative processes." He goes on to quote Wolf in *Essentials of Scientific Method* 1928 "…This should drive home the fact that even our so-called definite statements of identification are actually statements of probability."[1]

To further understand the level of usage in the community of the current published standard, a survey was developed to ascertain the adherence to the standard and also to determine how many of the varying six qualified opinions are being used by each surveyed laboratory or consultant. Alternatives to the scale, limiting some of the qualified opinions, were also proposed via sample cases wherein examiners were asked to choose one of two opinions and to state the basis and reasons for their choice. This provided information for a process whereby some terms could be edited to be defined more specifically or some, possibly, eliminated.

Feedback on the use of language derived from likelihood ratios whereby propositions both for and against differing hypotheses was obtained. These ratios reflect the truthfulness of differing propositions and how strongly one proposition is supported by the evidence over the other. The comments on this language will be provided and may be applied to discussions within the National Institute of Standards and Technology (NIST) -sponsored OSAC Questioned Document Opinion Terminology task group. It may, in turn, be disseminated to associated task groups in other OSAC forensic subcommittees.

Consistency of reporting among all forensic specialties has been the subject of many who have studied the needs of forensics to include those who authored the National Academy of Sciences Report in 2009. The difficult task of all specialties is to develop concise reporting language that is not only easily understandable but also adequately conveys the scientific foundations that support them. This project may provide some of the needed groundwork in the Questioned Document discipline to reach that goal.

Reference(s):

1. A. Wolfe, Essentials of Scientific Method, George Allen & Unwin Ltd., London, 1928

Questioned Documents, Opinion Terminology, OSAC

J8 The Impact of *Daubert* on Forensic Document Examinations — The Paradigm Shift

Jan S. Kelly, BA, 9360 W Flamingo Road, #110-400, Las Vegas, NV 89147*

After attending this presentation, attendees will have a better understanding of the impact of the Supreme Court's *Daubert v. Merrell Dow Pharmaceuticals, Inc.* 1993 decision on all of the sciences, including forensic document examination, and how this impact set in motion a paradigm shift in testimony, testing, and academic research.

This presentation will impact the forensic science community by educating forensic document examiners as to the steps taken between 1993 and 2015 to document the reliability of forensic document examination and how the field satisfies *Daubert*.

Prior to the *Daubert* decision in 1993, the courts relied on Rule 702 in the Federal Rules of Evidence and on Frye's general acceptance standard to determine whether an expert would be allowed to testify. In *Daubert*, the Supreme Court established new guidelines for the admissibility of scientific evidence using five general guidelines: (1) whether a theory or technique was tested (falsification); (2) whether there are standards controlling the technique's operation; (3) whether there is a "known or potential error rate of the technique"; (4) whether the theory or technique has been subjected to peer review and publication; and, (5) whether or not the technique or theory has general acceptance in the scientific community.[1] These criteria created a paradigm shift in the content of testimony as they now required the scientist to testify to the reliability of the methodology.

Efforts to prove the reliability of forensic document examination to the courts included the Forensic Document Examiner (FDE) community participating in a series of blind proficiency tests administered by Dr. Moshe Kam, a Professor at Drexel University, School of Engineering and Applied Sciences. FDEs participated in five tests between 1994 and 2003. The results of the Kam tests verified that forensic document examiners reliably and significantly outperform laypersons in tasks involving handwriting comparisons. American Standards Testing and Materials (ASTM) was chosen as the publisher of FDE standards that control a technique's operation. The standards published by ASTM provide objective documentation that the profession has standards in place that have been reviewed by both the FDE and other scientific communities. The FDE community partnered with academia to conduct several research projects funded through the National Institute of Justice (NIJ) to further establish the reliability of forensic document methodologies.

This presentation will enable the attendee to understand the progress made by FDEs following the promulgation of *Daubert* in providing and documenting the reliability of the examiners and their methodologies in order to assure the courts that forensic document examination meets every one of the five *Daubert* factors.

Reference(s):

1. *Daubert v. Merrell Dow Pharmaceuticals Inc.* (92-102), 509 U.S. 579 (1993)

Daubert, **Paradigm Shift, Reliability**

J9 Forensic Document Examination by a Multispectral Mobile Forensic Imaging System

Halis Dokgöz, Mersin University Medical Faculty, Dept of Forensic Medicine, Mersin 33100, TURKEY; and Hakan Kar, MS*, Mersin University Medical Faculty, Forensic Dept, Mersin 33100, TURKEY*

After attending this presentation, attendees will have a greater understanding of a multispectral mobile forensic imaging system for detecting originality, alterations and distortions, or any modifications on questioned documents as a basic screening test.

This presentation will impact the forensic science community by illustrating the efficiency of using different polarized or non-polarized imaging on questioned documents.

Forensic document examination is one of the most important fields of forensic sciences. Questioned documents can be examined for evidence of alteration, obliteration, erasure, and page substitution. The examiner can use various methods, materials, or machines that created the documents, providing key information that can identify or narrow the possible sources of the document. The ink, paper, writing tools, ribbons, stamps, and seals used in production of the document may all reveal important clues. The examiner may even detect valuable evidence in a document's invisible impressions.1 Special techniques such as photography, microscopy, lighting, electrostatic detection apparatus, and chromatography are used in different qualifications in forensic document examination.2 This study tests the usefulness and efficiency of a multispectral mobile forensic imaging system, ForenScope®, on detection of originality, alterations and distortions, or any modifications of the questioned documents.

ForenScope® is a system that turns a smartphone, a mobile device with a camera, or a tablet into a mobile multispectral imaging system with a 200nm-1,000nm imaging band coupled with the ability of recording and transferring. **ForenScope®** provides polarized/non-polarized imaging at the same time with a very different method using soft white Surface-Mounted-Device Light-Emitting Diodes (SMD LEDs) integrated to the standard system.3 ForenScope® was tested on questioned documents that contained evidence of false identities, counterfeit money, false checks, falsification of documents, chemical process, adding extraction, and consecutive overlay. Polarized and non-polarized macro and micro lenses were tested with different light sources (from 365nm Ultraviole to Dark Red) for imaging to determine the best combination setting for each document. Results of the study reveal the advantages and limitations of ForenScope®.3

During the study, it was condluded that ForenScope® is portable, easy to use, consistent with smart phones and tablets, and displays the features of fast imaging, sharing and archiving, and high-quality distant or macro imaging that result in successful polarized imaging features. In addition to these advantages, dependency on the technical capabilities of the smart phone's or tablet's camera is a major limitation.

As a result, a multispectral mobile forensic imaging system such as ForenScope® is a portable device that can be used as a basic screening test for detecting originality, alterations, distortions, or any modifications to a questioned document.

Reference(s):

1. A Simplified Guide To Forensic Document Examination. http://www.crime-scene-investigator.net/SimplifiedGuideQuestionedDocuments.pdf Access date: 07.28.2015.
2. Guide for the development of forensic document examination capacity. United Nations New York, 2010.
3. Meet with a New Concept It's the First Multispectral Mobile Imaging and Archiving System in the World. http://www.forenscope.com/icerik/products_forensic.html Access date: 07.28.2015.

Forensic Document, Forenscope, Multispectral Mobile Imaging

J10 Developing an Ink Database for Commonly Used Pens Manufactured in Pakistan

Zumrad U. Bhutta, MS, Chak 84 South Branch Sargodha, Sargodha, Punjab 40100, PAKISTAN; and Ayesha Imtiaz, MS*, Government College University, Nano-Chemistry Lab, GC University Lahore, Katchery Road, Lahore, Punjab 54000, PAKISTAN*

The goal of this presentation is to present one of the first important research projects from Pakistan regarding database inks.

This presentation will impact the forensic science community by detailing the steps taken to establish a database of inks used in ballpoint pens, gel pens, and other types of pens in Pakistan where forensics is a new field.

Pakistan is a developing country where questioned document laboratories are very limited as forensics is a new field. These forensic laboratories encounter a variety of cases that are received from all regions of Pakistan. In this study, the database of different brands of blue and black ballpoint pens, gel pens, and other types of ink pens used in Pakistan were established. The variations in blue and black inks were observed because of the presence of different colorants. The data of Ultraviolet/Visible (UV/Vis) spectrophotometer and Fourier Transform Infrared (FTIR) will enable questioned document experts to recognize which brand of ink was used to forge the document, making discrimination and comparison concerning addition, alteration, or obliteration in the document easy to reveal. In this study, the database of different brands of commercial ink pens are further used for comparison of different questioned documents such as bank checks, wills, court orders, and property documents. This presentation will impact the forensic science community by providing a sufficient amount of data that is required to process the questioned documents more efficiently by being less time consuming and less expensive.

In many criminal and civil cases in Pakistan, the most commonly questioned documents are those written in pen ink. An important task for forensic document examiners is to identify whether two or more ink entries on one or more documents were written with the same ink type. Comparison analysis of the ink reveals information about addition, alteration, or obliteration of entries on the document. In this study, a wide range of commercial blue and black ballpoint pens were used to investigate the discriminating characteristics of the different inks found on the same document. The ink from pens and ink extracted from lines on paper written with ballpoint pens were subjected to UV/Vis spectroscopy, Infrared (IR), and reflectance spectrophotometer.

Questioned Documents, VSC 6000/HS, FTIR

J11 Conductive Inks: Implications for Forensic Document Examiners

Kevin P. Kulbacki, MSFS, Osborn & Son, 1273 Bound Brook Road, Ste 15, Middlesex, NJ 08846*

After attending this presentation, attendees will have a greater understanding of conductive inks. This presentation will explore their potential uses, their unique properties, and their implications for the various techniques used in forensic document examination.

This presentation will impact the forensic science community by providing subjective information about conductive inks and the actual and theoretical implications that may be associated with their presence on questioned documents.

Conductive inks have been publicly available for multiple years; however, they have had numerous disadvantages that limited their practical use. Early conductive inks have suffered from the inefficient transporting of electrical currents, hours of drying time required, and the need for a stiff base to which they must be applied. Due to the limited potential for practical implications in casework, forensic document examiners and researchers have not studied conductive inks in depth.

Recently, a company has developed efficient water-based conductive inks that are deposited using a ballpoint pen. These inks were developed as an educational tool for students learning basic circuitry. Through application with an ordinary ballpoint pen and the application of magnetic attachments, it is possible to create a working circuitry encompassing switches, resistors, and lights on a piece of paper. Despite their intended use, there are numerous potentially nefarious uses of this technology that could lead to an examination by a forensic document examiner. Due to these potential uses, earlier research presented at the 2015 American Academy of Forensic Sciences Annual Scientific Meeting has been further researched and expanded upon to provide more in-depth information about this technology.

Conductive inks pose unique theoretical implications for the forensic document examiner. The development of indentations with electrostatic detection devices relies upon detecting different charge densities on a questioned document. Conductive inks, unlike ordinary inks, are designed to attract a charge. It is therefore theoretically possible that the charge density of the questioned document may be affected in a manner that is adverse to the development of indentations. This presentation will attempt to provide a more subjective understanding of what effect, if any, there is on the electrostatic detection of indentations.

The previous research presented on conductive inks was specifically focused on its effects on the electrostatic detection of indentations on a questioned document. This updated presentation also includes a systematic examination of conductive inks including numerous techniques to provide a more comprehensive picture of what a forensic document examiner could expect to see when conducting an examination of conductive ink.

Conductive, Ink, Documents

J12 A Comparative Study of Common Individual Writing Characteristics in Determining Left- and Right-Hand Writings

Vikram Raj Singh Chauhan, PhD, Patiala Bureau Of Identification, 2460/1, Shamsher Singh Street, Near Triveni Chowk, Patiala, Punjab 147001, INDIA*

The goal of this presentation is to distinguish one handwriting from another and to determine handedness of the writer.

This presentation will impact the forensic science community by showing how individual writing characteristics are useful in determining the handedness of the writer.

The majority of the world's population, irrespective of gender, is accustomed to writing with the right hand; few people use their left hand and very rarely a person is ambidextrous (i.e., is able to write with both hands). The determination of the handedness of a suicide note and of anonymous, threatening, and disguised writings is a vital point of inquiry for a handwriting expert. Handwriting is not only a design of letters, but also of specific mannerisms of writing based on the individual writing characteristics which help to associate a writer to a handwritten text. Certain individual writing characteristics are more often associated to either left-handed or right-handed writing that would allow the document examiner to determine the handedness of the writer.

The present study was conducted using a total of 500 writing samples from male and female writers who were between the ages of 18-65 years. The background of the writers varied and included students, retirees, and those employed in either the public or private sectors. The 500 samples were evenly divided into 250 left-handed and 250 right-handed writers whose writing was used to categorize individual writing characteristics more commonly associated to the left- or right-hand writing. For comparative study of Roman script, in upper and lower case, and numbers zero to nine, four writing samples were taken from the paragraphs in the Huber and Headrick *Facts and Fundamentals* book.[1] Using gel and ballpoint pens on white executive bond paper, the text was dictated to each writer who was using his or her accustomed writing hand. The writing samples were critically and exhaustively examined to differentiate the common individual writing characteristics that can be used in determining whether the writer was left- or right-handed.

Reference(s):

1. Roy A. *Huber*, A.M. *Headrick, Facts and Fundamentals,* CRC Press; 1st edition, April 15, *1999*

Handedness, Characteristics, Accustomed

J13 Security Feature Implementation: The Other Side of Document Security

Joel A. Zlotnick, MSFS, U.S. Department of State, 600 19th Street, NW, Ste 12.601, Washington, DC 20522*

After attending this presentation, attendees will gain an understanding of why counterfeit deterrence is not just a function of security feature selection. In fact, the resistance of a security document to physical fraud also depends on how security feature technologies are integrated into the overall document design.

This presentation will impact the forensic science community by contrasting implementations of specific security feature technologies in documents such as passports, visas, identity cards, birth records, and currency. Attendees will develop a greater understanding of how to assess the value of security feature technologies based on how they are used and will be able to more critically evaluate feature authenticity using design considerations.

How should counterfeit deterrence be assessed? Some issuing authorities view document security as just a checklist, where a document must contain a minimum quantity of security features to meet a specified security threshold, but little attention is given to how the technologies interact with one another to make the document more robust than the sum of its parts. The effectiveness of a security feature depends on many factors: (1) the availability of the technology in commercial markets; (2) ease of feature recognition and inspection by both trained and untrained viewers; and, (3) how the feature is integrated into the document.

Recent decades have seen an explosion in the quantity and quality of security feature technologies available for prevention of physical attacks on security documents. Novel technologies that are new to market, or which possess limited commercial applications, are often attractive as new security features because they are relatively inaccessible to counterfeiters. As security feature technologies mature, many find applications in the commercial world, making them more available to criminals and potentially reducing their intrinsic value as security technologies. This view of security features as possessing a life span has merit and should not be ignored, particularly in light of many documented instances of complex security feature technologies (or high-quality simulations of them) being detected on counterfeit documents or sold into commercial markets over the internet. Specific examples will be provided.

If the above viewpoint holds that it is the rarity and proprietary nature of a security feature technology that provides its value for counterfeit deterrence, consider an opposing viewpoint: it is also the specific implementation of a technology, and not just the technology itself, that deters counterfeiting. For example, offset printing is widely used in the commercial world, yet it also finds broad applicability in production of passports, visas, birth records, identity cards, and other documents. Similarly, Ultraviolet (UV) -reactive and other specialty inks have been available in commercial markets for years, yet inclusion of UV-responsive artwork is nearly ubiquitous in security printing and UV inks are not considered high-security materials.

What these two examples have in common is that it is not the technology itself that provides security, but rather how it is employed differently in security environments than in commercial environments. In the offset printing example, commercial offset typically uses dot halftones and process color, so security document artwork is created almost exclusively using line art and spot color for no other reason than to make it different from commercial offset printing. Regarding UV inks, it is not the presence or absence of a UV response that makes a document genuine or counterfeit. Instead, genuine document issuers rely on intricate design work and the use of specialized printing techniques to create complex UV designs that counterfeiters may not possess the skill to replicate convincingly. The common thread between these two examples is that it is design, not just fundamental characteristics of the technology, that makes it appropriate for use in security printing.

The message from these and many other examples is that viewing a security feature technology as either secure or not secure ignores implementation considerations. This presentation argues that assessing a broader range of security technologies in terms of how they are implemented clarifies how these technologies can best be used in security environments and even prompts new security applications for certain document components that are often regarded as low-security commercial materials.

Security Feature, Counterfeit, Design

J14 The Value of Skill Task Assessments (STA)

Jan S. Kelly, BA, 9360 W Flamingo Road, #110-400, Las Vegas, NV 89147*

After attending this presentation, attendees will have a better understanding of STAs. Attendees will also gain an understanding of the similarities and differences between STAs and existing historically utilized proficiency testing programs.

This presentation will impact the forensic science community by demonstrating the impact and benefits that skill task testing can provide as a training tool and mechanism to periodically self-assess and evaluate an individual or group level. Repeated participation in STAs also provides a means to document the reliability of specific methods of analysis in forensic document examination.

STAs are practical exercises that provide additional training and a measure of the examiner's skill on a specific examination task. The purpose of a proficiency test is to test the examiner's ability to examine a "case" that may include one or more document examination methodologies. STAs typically focus on one task and are designed to provide the opportunity for the Forensic Document Examiner (FDE) to work an extensive training exercise that allows the participant to evaluate his/her reasoning process to the chosen range of conclusions. STAs are also another tool for documentation that support the reliability of forensic document examination methodologies.

STAs are blind assessments that can be used for training purposes or for documentation of an individual error rate. The forensic document examination task of determining whether signatures were genuine, spurious, disguised, or simulated has been the focus of STAs offered by LaTrobe University and Skill-Task Assessment Training and Research Inc. (ST²AR). Once testing is closed, each participant receives a Post-Test Information Package (PTIP) containing test results that include the group error rate and the participant's individual error rate (if the answer sheet is returned). The PTIP allows the participant to self-evaluate his or her performance in comparison to other participants. It also provides feedback since there is a true known answer for each questioned signature. Whether the participant voluntarily submits his/her answer sheet for evaluation or uses the test for self-assessment, STAs allow the participant to adjust the "calibration" in their decision-making process with the opportunity to re-evaluate their choice of opinions for each signature.

This presentation will assist the attendee in understanding the differences between skill task assessments and proficiency tests. The design of STAs allows the examiner to complete an extensive training exercise on a single task and to evaluate his or her reasoning process to the chosen range of conclusions. STAs also further establish the reliability of forensic document examination methodologies.

Skill Task Assessment, Proficiency Tests, Reliability

J15 A Triad of Techniques and Instruments for the Examination of Questioned Documents

F.L. Jim Lee, Jr., MS, Summit Forensic Document Examination Lab, PO Box 207, Eden, UT 84310*

After attending this presentation, attendees will better understand the techniques and instruments available to the questioned document examiner and will be updated on their current status.

This presentation will impact the forensic science community by alerting the community to the advances made in three instruments used to aid in questioned document examination, namely, the FORAM® Raman spectrometer, the Elemental Composition Comparator (ECCO), and the Video Spectral Comparator (VSC®) 8000.

Additionally, this presentation is intended to provide attendees with an update on new instrumentation for forensic document examination, available from Foster + Freeman as an update regarding ongoing research with these instruments.

Visual Examinations: The VSC® 8000 combines sophisticated digital imaging and multi-wavelength Light-Emitting Diode (LED) technology with a clear and efficient software interface to provide a complete capability to the examination of questioned documents in order to reveal hidden details and irregularities such as alterations, obliterations, additions, and deletions, depicting them in Ultra High Definition (UltraHD) as sharp, bright images using super resolution imaging and displaying them on a 4K UltraHD monitor.

Advanced Instrumental Analysis: Beyond the visual examination of documents and their security features, it is possible to probe deeper into the chemical makeup of a document, revealing information about the origins and elemental composition of different paper and ink types and revealing the latest generation of embedded microscopic security taggants. There are two analytical instruments available which allow for this type of analysis. The first is the FORAM® Raman spectrometer 785nm; the Raman spectrometer is used for the examination of ink, toners, and other materials attached to documents. Raman spectra exhibit numerous features that are specific to molecular structure and provide valuable "signatures" for comparing and differentiating materials, making it an ideal technique for examining ink and other materials attached to documents. The FORAM® is available with a choice of three laser wavelengths: 785nm, 685nm, and 532nm. High levels of sensitivity can be achieved with the 532nm laser, while the 785nm infrared laser is better able to suppress fluorescence. A highly stable 685nm red laser provides a third option. FORAM® instruments have an integral video microscope to assist sample selection, a large XYZ translation stage and dedicated software for analysis and database comparison. The second instrument is the ECCO which employs the use of Laser-Induced Breakdown Spectroscopy (LIBS) for the elemental analysis of evidence, providing elemental analysis on materials as small as 300 microns. With a large sample chamber, ECCO is designed for the analysis of paper, glass, metals, paint, fibers, minerals, and gunshot residues. The system uses a high-intensity pulsed laser focused onto the sample to create a plasma of vaporized matter that emits an atomic spectrum of the constituent elements. A database of emission lines provides automatic identification and labeling of elements present. Features include rapid analysis and automatic identification of elements requiring only minimum sample preparation.

Document Examination, Ink Examinations, Applications

J16 How Well Do People Know Their Signatures?

Zuzanna Kazmierczyk, BS, Department of Natural Sciences, University of Derby, Kedleston Road, Derby DE22 1GB, UNITED KINGDOM; and Ian J. Turner, PhD, Department of Natural Sciences, University of Derby, Kedleston Road, Derby, Derbyshire DE22 1GB, UNITED KINGDOM*

After attending this presentation, attendees will be aware of the ease and effectiveness with which one can forge a signature using a simple method. Attendees will also learn the challenges of differentiating a genuine signature from a simulated signature by the original author of the signature.

This presentation will impact the forensic science community by showing that, with the use of a simple device and computer programs, forged versions of signatures can be easily produced. It will show Forensic Document Examiners (FDEs) that differentiating between genuine and simulated signatures is complex and sometimes even the author can be unaware of the nature of their own "signature."

Signatures have always served the function of identifying a writer and verifying or authenticating documents. The act of writing a name is an automatic action and can become extremely individualized. Therefore, one can assume that a signature itself can, or even should, be highly specific and unique for every individual. This could imply that each person should be familiar with his or her own signature and thus be able to discriminate between the genuine signature and a simulation.

The purpose of this study was to investigate how well people know and recognize their signatures. One hundred participants were asked to provide ten signatures on paper. The sheet was then scanned to obtain an "electronic" version. The original genuine signatures on paper served as a model to produce simulated versions on a computer (with the help of a digitizing tablet). Then both groups of signatures (scanned genuine and computer-processed simulations) were adjusted in order to make them the same size. After two weeks, every participant was shown ten signatures one by one on a computer screen and asked to judge whether they were genuine or forged (for every participant, a set of ten signatures shown to them contained from zero to ten simulated signatures).

The results show that it can be difficult for people to recognize their own genuine signatures and differentiate them from the forged versions. Everyone found the task challenging, even those who claimed to have had characteristic and "unforgeable" signatures. Among all 100 participants, only one person was 100% correct in recognizing the signatures (ten out of ten signatures were judged correctly to be either genuine or simulations). The lowest result was 20% (two out of ten correct answers) and the average of all results was 57.6%. The total number of questioned signatures in the project was 1,000 (100x10). Out of 550 genuine signatures, 309 (56.2%) were correctly recognized to be genuine. Out of 450 simulated signatures, 267 (59.3%) were correctly identified as simulations. Only 25 participants correctly identified all forged versions of their signatures (eight of these participants had only one forged signature in the set of ten signatures that were presented to them).

Signature Forgery, Simulated Signatures, Genuine Signatures

J17 Properties of Inkless Pens

Samiah Ibrahim, BSc, 139 Riverdale Avenue, Ottawa, ON K1S 1R1, CANADA; and Tobin A. Tanaka, BS*, Canada Border Services Agency, Government of Canada, Ste 280-14 Colonnade Road, Ottawa, ON K2E 7M6, CANADA*

After attending this presentation, attendees will better understand the detailed properties of inkless pens on different substrates (paper types).

This presentation will impact the forensic science community by explaining the findings of research into the macroscopic, microscopic, physical, and chemical properties of one type of inkless pen.

The current iteration of the "inkless pen" is not really new at all, but is rather a type of writing instrument that relies on metalpoint. Metalpoint is the classic drawing technique that uses a sharpened metal rod or wire to make the visible lines on a writing surface, typically paper or parchment. This visible line is the result of metallic deposition of material on the page. The metal was typically lead, silver, copper, or gold, although silver was most the common and "silverpoint," as it is called, has achieved popularity as an art form many times during the past 600 years. The new inkless pens are marketed not so much for artistic purposes but for general use on a variety of surfaces.

Forensic Document Examiners (FDEs) must be aware of such writing instruments in both the historical and present-day context. In this research, the detailed physical and chemical properties of one brand of inkless pens and their written line are explored.

Various paper types were written upon to determine what effect different materials would have on the appearance and morphology of the written line. These paper types included assorted wood fiber writing papers, assorted non-impact printing papers, cotton content writing paper, newsprint, mixed fiber boxboard, stone paper, wax paper, thermal paper, and water-resistant paper. Examination of the written line was accomplished with the unaided eye and via stereomicroscopy with surface profiling by confocal microscopy. A descriptive assessment of the written line on these different substrates is provided with some insights into which of these substrates are not as suitable and the potential reasons for this. Additional examination of the written lines was conducted by radiographic and analytical chemical methods for inorganic and elemental composition.

The resistance of the written line to erasure and alteration on these different paper types was also explored. Erasure was attempted by hand-held erasers, chemical attack, and laser ablation. A qualitative assessment of the degree of "inkline" removal was made. Analysis also sought to determine whether any alterations to entries could be detected by radiographic methods, given the composition of the writing line as determined by various analytical methods. This approach differs from previous work which explored whether spectral differentiation of the written lines from inkless pens could be achieved.1

Finally, a detailed examination of the indentations produced by these inkless pens on the various substrates was conducted. These examinations included simple indentations as well as the intersections of the written line with already-present indentations.

While this analysis and reporting is comprehensive for one brand, it cannot be extrapolated to other brands of metalpoint, inkless writing instruments. This research serves to educate the FDE community and to explore options for analysis in the event one is confronted by this technology during the course of casework.

Reference(s):

1. Gardner, M. Inkless Pens? *Proceedings* of the American Society of Questioned Document Examiners, 2015, Toronto, Canada.

Writing Instrument, Inkless Pen, Document Examination

J18 The Leon Savoy Estate

David S. Moore, MEd, 9010 Barrhill Way, Fair Oaks, CA 95628*

The goal of this presentation is to provide attendees with an interesting case involving a holographic will in which two opposing forensic document examiners with different training backgrounds arrived at entirely different opinions. The various aspects of the will that were considered will be discussed and demonstrated.

This presentation will impact the forensic science community by discussing alternate explanations for two examiners who were provided the same evidence arriving at diametrically opposing findings.

This case example involves the holographic handwritten will of Leon Charles Savoy, Jr., and the various forensic document examinations that were conducted to determine the authenticity of this will.

In this matter, there were two opposing forensic document examiners who arrived at findings totally opposite from one another. This present case is similar (in a variety of ways) to a paper presented several years ago in which there were also diametrically opposing opinions by different examiners.

When forensic document examiners arrive at completely different opinions, it is a legitimate endeavor to explore why this happened and whether formal training — or the lack thereof — may be an underlying cause for these differing opinions. In the present case, one examiner had been formally trained in the mid-1970s in accordance with the requirements outlined in American Society for Testing and Materials (ASTM) Standard E-2388 (Standard Terminology for Expressing Conclusions of Forensic Document Examiners), which requires, among other things, a minimum of two years of formal training in this field. The opposing examiner was primarily self-trained and such informal training did not meet the requirements of this ASTM Standard (indeed, the opposing examiner in this case, as was the situation in the previously presented case, was originally trained as a graphologist). Furthermore, one examiner had been certified by the American Board of Forensic Document Examiners (ABFDE) since it's inception in 1978, while the opposing, less-formally trained examiner was certified by the National Association of Document Examiners (NADE). In many ways, this case is another perfect example as to why formal training should be a prerequisite for individuals who claim expertise in this field.

The questioned will in this present matter was three full pages of handwriting, followed by the purported signature of Leon Savoy, Jr. In the opinion of one examiner, the handwriting and signature in the questioned will were rapidly and skillfully written, while the opposing examiner arrived at a different opinion: in his view, the questioned writing was "slow," "tangling," and "nearly erratic."

Both examiners were allowed to evaluate the original of the questioned will in their respective laboratories. Furthermore, each examiner was provided with large quantities of contemporary, genuine signatures and known handwriting. In other words, the conditions for a complete and thorough examination of the evidence in this case were ideal. Yet, these examiners, each with more than several decades of experience, arrived at totally different opinions: one concluded that the questioned will was written and signed by Mr. Savoy while the other concluded the exact opposite.

The will, which apparently left the vast majority of Mr. Savoy's estate to a female acquaintance, was subsequently challenged by relatives who were excluded from the will. The opposing opinions in this case set the stage for a protracted will contest in which each examiner provided several bouts of lengthy deposition testimony. Ultimately, the matter resolved before trial.

It was suggested that both examiners present their positions in back-to-back papers to the American Academy of Forensic Sciences; however, the opposing counsel refused permission for his examiner to participate.

This handwriting and signature case involves considerations of simulation, tracing, alterations, erasures, indentations evidence, ink and pencil writing, and other important aspects that, ultimately, led to a determination of the authenticity of the will. Often, holographic will contests involve only one or a few of these considerations; however, as with the previous case, this case involved all of these and more. Attendees will be provided with the overwhelming evidence that was available for consideration, all of which led directly to the determination of genuineness.

This presentation demonstrates the relevant aspects that were considered and explored in the examination of this will. While each of these aspects directly addressed the issue of the will's authenticity, the opposing examiner apparently either did not consider them or consciously discounted them. Ultimately, the question must be asked: Was the opposing examiner's opinion based upon poor training, an inability to properly evaluate the significance of the overwhelming evidence, or were there ethical considerations involved?

Holographic Will, Handwriting, Document Examination

J19 Status of the Expert Working Group on Human Factors in Handwriting Examinations

Thomas W. Vastrick, BS, 522 S Hunt Club Boulevard, Ste 217, Apopka, FL 32803*

After attending this presentation, attendees will have an understanding of goals and objectives of this National Institute of Standards and Technology (NIST) -administered project.

This presentation will impact the forensic science community by providing details of what can be expected in the near future as it relates to forensic document examination and this discipline's responses to recent recommendations for strengthening the field.

In response to the 2009 National Academy of Sciences (NAS) Report, Strengthening Forensic Science in the United States: A Path Forward, the forensic document profession has been given a portal view of this profession's future. To say that forensic document examination picked up the ball and ran with it would be an understatement. The production of working standards, the publication of numerous significant research projects concerning the foundation of scientific methodologies, and a near-total review of the forensic document examination profession has rendered practitioners well-prepared to face the challenges that are rising with the advent of new forms of criticism and attack.

One such project is the formation of a blue-panel committee of experts in forensic document examination, statistics, research, academia, and the legal profession to come together as one to map the structure under which forensic scientists will practice for decades to come.

What are human factors? These are simply the by-product of people being people. Understanding human idiosyncrasies allow the design of methodologies of examination that can minimize the potential of error and strengthen efficiency and accuracy of forensic scientists.

This presentation will discuss human factors, scope of work for the committee, how this work affects the forensic science practitioner, and what the attendees would like to see as a result of this project. Included in the presentation will be a discussion period in order to obtain the thoughts and ideas of those outside the committee that may assist committee members in the development of the final report.

The Expert Working Group (EWG) Committee has subcommittees and members of other subcommittees who may be called upon during this presentation. There will be some limitation as to what can be discussed outside of the committee, but this presentation will highlight the general goals of the project and overall subject thoughts in addition to some personal thoughts.

Human Errors, Handwriting, NIST

J20 Critics Say the Darndest Things!

Jan S. Kelly, BA, 9360 W Flamingo Road, #110-400, Las Vegas, NV 89147*

After attending this presentation, attendees will better understand the impact of criticisms against forensic document examination by members of academia. Statements made during testimony by the critics and how the forensic document community responded to the criticisms will be discussed.

This presentation will impact the forensic science community by educating the Forensic Document Examiner (FDE) as to statements made during a critic's testimony of forensic document examination and how this criticism influenced the examiner's court preparation.

Academia entered the forensic science arena as critics against specific forensic science professions in the late 1980s. Initially, criticisms appeared in venues that do not offer forensic document examiners the same level of access given to academia, such as law review journals and speaker presentations at judicial conferences. The forensic document profession was one of the first forensic sciences to attract academic critics. By the late 1990s, critic attacks on forensic science expanded to latent prints, firearms, and other forensic science disciplines. Not only did the number of disciplines coming under fire by academic critics increase, opportunities for the critics to express their criticisms also increased as they began appearing in the courtroom as defense witnesses.

Testimony offered by university professors against FDEs presented a unique situation during a time when the courts were adjusting to their gatekeeping role of incorporating the Daubert five-prong test instead of *Frye's* general acceptance criteria. Rule 702 in the Federal Rules of Evidence states: "A witness who is qualified as an expert by knowledge, skill, experience, training, or education may testify in the form of an opinion or otherwise if: (a) the expert's scientific, technical, or other specialized knowledge will help the trier of fact to understand the evidence or to determine a fact in issue; (b) the testimony is based on sufficient facts or data; (c) the testimony is the product of reliable principles and methods; and (d) the expert has reliably applied the principles and methods to the facts of the case."1 The primary issue the courts faced was that the critic did not present himself as an "expert" in the field he was criticizing, but as a "friend of the court." The critic's testimony focused on the premise that FDEs could not prove the main tenets of the document discipline. Being challenged by a witness whose only knowledge of the field was from reading Albert Osborn's treatise Questioned Documents and Ordway Hilton's Scientific Examination of Questioned Documents was difficult because the critic's interpretation of the treatises was inaccurate. Admittedly, FDEs were not prepared to respond to an attack that was summarized with the critic stating, "They can't prove it."

Transcripts of critic testimony revealed numerous inaccurate statements made by FDE critics. This presentation will discuss the inaccuracies of critic testimony as revealed in various transcripts. The presentation will also discuss how FDEs revised the content of their testimony as a way to address the criticisms offered by the defense critic. The journey in learning how to communicate the reliability of forensic document examination to the courts was a lengthy process, but necessary because critics say the darndest things!

Reference(s):

1. Federal Rules of Evidence. Accessed on July 31, 2015 from https://www.law.cornell.edu/rules/fre/rule_702

Forensic Document Examiner, Critic, FRE 702ts

TOXICOLOGY

K1 Driving Under the Influence of 5-MAPB: A Case Report

Brittany Thomas, MFS, Washington State Patrol Toxicology Laboratory, 2203 Airport Way, S, Ste 360, Seattle, WA 98134; Lisa Noble, BS, Washington State Patrol Toxicology Laboratory, 2203 Airport Way, S, Ste 360, Seattle, WA 98134; Brianna Peterson, PhD, 2203 Airport Way, S, Seattle, WA 98134; and Fiona J. Couper, PhD, Washington State Patrol, 2203 Airport Way, S, Ste 360, Seattle, WA 98134*

After attending this presentation, attendees will better understand the novel psychoactive substances 5-APB and 5-MAPB and their effects on driving performance. An impaired driving case is presented in which 5-MAPB was the only drug detected and the observations made by the arresting officer suggested the presence of a stimulant drug.

This presentation will impact the forensic science community by providing law enforcement, toxicologists, and those in the medical community with observations of an individual under the influence of 5-MAPB and detection methods used to screen for 5-MAPB in whole blood.

5-MAPB is the N-methyl derivative of 5-APB. Both compounds are analogues of amphetamine and methamphetamine and are considered novel psychoactive substances. Their benzofuran ring also makes them analogues of MDA and MDMA. Similar to MDA and MDMA, they are consumed as stimulating or entactogenic drugs with euphoric and empathogenic effects.[1,2] Sold as so-called "research chemicals" via the internet, these compounds first appeared in 2010 and 2012, when users started discussing their effects on internet drug forums.

In this case, a 19-year-old male was stopped at 3:00 a.m. for driving erratically. He reportedly was drifting back and forth across the center skip line and the fog line. When he was contacted by the officer, he indicated the reason for his poor driving was that he and his passenger were in a "heated debate and he was distracted." The subject stated he was coming from a concert and had worked a 19-hour day.

According to the officer's report, the subject was very energetic, unable to hold still, talked excessively, and spoke very quickly. His eyes were reportedly wide open, his pupils were dilated, and didn't react to the officer's flashlight. The subject reportedly admitted to using heroin, marijuana, and an unknown anti-psychotic medication.

Ethanol and immunoassay drug screens were negative. Routine testing for stimulant drugs and opiates were also negative. A basic drug screen by Gas Chromatography/Mass Spectrometry (GC/MS) revealed the presence of a significant peak (RT=7.453min) with the following (m/z) fragments: 58 (base peak), 131, 77, 102, 42, 174, 207, 327. A similar fragmentation pattern was observed in the Cayman Spectral Library for the compound 5-MAPB. Further confirmation testing was performed using liquid-liquid extraction followed by derivatizion with heptaflurobutyric anhydride; comparison of a purchased standard to the unknown sample confirmed the presence of 0.44mg/L of 5-MAPB in the blood by GC/MS Selected Ion Monitoring (SIM).

Limited information about 5-APB and 5-MAPB exists in the literature, particularly in relation to impaired driving. Knowledge of the effects of these drugs is mostly limited to self reports by users on online discussion boards.

Reference(s):

1. Welter J., Kavanagh P., Meyer M.R., Maurer H.H. (2015). Benzofuran analogues of amphetamine and methamphetamine: studies on the metabolism and toxicological analysis of 5-APB and 5-MAPB in urine and plasma using GC-MS and LC-(HR)-MS^n techniques. *Anal Bioanal Chem*. Doi:10.1007/s00216-014-8360-0
2. Welter J., Brandt S.D., Kavanagh P., Meyer M.R., Maurer H.H .(2015). Metabolic fate, mass spectral fragmentation, detectability, and differentiation in urine of the benzofuran designer drugs 6-APB and 6-MAPB in comparison to their 5-isomers using GC-MS and LC-(HR)-MS^n techniques. *Anal Bioanal Chem*. Doi:10.1007/s00216-015-8552-2.

5-MAPB, Designer Drugs, DUID

K2 Incidence and Trends of Driving Under the Influence (DUI) of Zolpidem: A Retrospective Study of DUI Cases From 2001 to 2014

Monica Jacobs, 12615 SW 256 Terrace, Homestead, FL 33032; and Lisa J. Reidy, PhD, Univ of Miami Forensic Tox Lab, 1600 NW 10th Avenue, RSMB R-5, Rm 7020A, Miami, FL 33136*

After attending this presentation, attendees will be informed of the incidence of DUI of zolpidem in DUI cases in Miami-Dade County, FL, from 2001 to 2014.

This presentation will impact the forensic science community by the increase and importance of zolpidem screening and confirmation in DUI cases.

Introduction: Zolpidem, currently a Schedule IV controlled substance under the Federal Controlled Substance Act, is not a controlled substance in Florida, rendering it difficult to charge drivers with DUI. The drug is an effective non-benzodiazepine sedative hypnotic which binds specifically to the $GABA_A$ receptor, which results in its sedative activity and therefore its central nervous system depressant properties. It has a rapid onset of action and short elimination half-life (average of 2.6 hours), which makes it ideal for the treatment of sleep disorders. Zolpidem may have a role in drug-impaired driving investigations since it has been shown that zolpidem can produce significant impaired coordinative, reactive, and cognitive skills such as erratic driving, slow and slurred speech, slow reflexes, disorientation, and confusion.

Objective: The purpose of this study was to investigate the trends of zolpidem use in drivers suspected of being under the influence, using biological samples (blood and urine) that had been submitted to the laboratory during the time period 2001-2014.

Materials and Methods: Blood samples from DUI drivers in Miami are first tested for the presence of alcohol by gas chromatography. In general, if a driver's Blood Alcohol content (BAC) is higher than 0.15g/100mL, no additional testing is performed. All urine and resulting blood samples are then tested for nine drug classes by Enzyme-Linked Immuno-Sorbent Assay (ELISA) (amphetamine (blood/urine cutoffs: 20/200ng/mL), methamphetamine (20/200ng/mL), benzodiazepine (20/100ng/mL), cocaine metabolite (20/150ng/mL), opiate (20/150ng/mL), oxycodone (20/100ng/mL), cannabis (2/20ng/mL), synthetic cannabinoids (20/10ng/mL), and zolpidem (5/25 ng/mL)) in blood and urine, respectively.

Urine and blood samples that screen as presumptive positive for zolpidem were subsequently confirmed by Gas Chromatography/Mass Spectrometry (GC/MS) using a basic drug screening and/or quantitated by utilizing a liquid-liquid alkaline extraction with analysis by GC/MS in Selective Ion Mode (SIM). The assay utilizes a five-point calibration curve (10-200ng/ml) alongside positive and negative controls. The method was validated in accordance with the Scientific Working Group for Forensic Toxicology (SWGTOX).

Results:

Year	Total number		% of cases
	Overall DUI cases	Zolpidem positive	
2001	885	3	<1
2002	946	3	<1
2003	700	5	<1
2004	653	4	<1
2005	520	4	<1
2006	522	4	<1
2007	647	9	1
2008	700	9	1
2009	640	14	2
2010	565	16	3
2011	506	9	1
2012	452	11	2
2013	403	8	2
2014	379	8	2

A total of 107 cases were positive for zolpidem during the 14-year period of this study. Overall, 80% of the zolpidem positive cases were identified in urine samples taken from suspected DUI drivers in Miami-Dade. The highest incidence of zolpidem positive cases was in the year of 2010 with a total of 16 samples. Blood quantitation values for zolpidem ranged from 12ng/mL to 560ng/mL (average=177ng/ml) in blood cases submitted.

*Presenting Author

Impairment profiles in the urine and blood cases in which zolpidem was detected included the presence of horizontal gaze nystagmus, lack of pupil convergence, reduced time estimation, and reduced pulse and blood pressure. Driving patterns ranged from falling asleep with the car stationary in the highway to the inability to maintain headway to failure to stop at intersections and collisions with oncoming traffic.

Conclusion: This study examined the incidence and trends of suspected human performance impairment cases involving zolpidem during 2001-2014. The incidence of urine DUI cases positive for zolpidem has increased over the past 14 years. The blood quantitation values demonstrated wide variability, with ten blood samples above the recommended therapeutic blood levels. Of interest, the United States Food and Drug Administration required lowering the recommended dose for zolpidem on January 13, 2013, due to the risk of next-morning impairment following its use. The failure to prosecute these cases in the state of Florida is of interest as zolpidem is shown to produce profound impairment in drivers under the influence of this medication.

Zolpidem, DUI, Incidence

K3 Retrospective of Phencyclidine (PCP) Incidence in Cleveland, Ohio, in Driving Under the Influence of Drugs (DUID) and Homicide Cases

Katherine Turner, 10548 Auburn Road, Chardon, OH 44024; Eric S. Lavins, BS, Cuyahoga County Medical Examiner's Office, Toxicology Department, 11001 Cedar Avenue, Cleveland, OH 44106; Rindi N. Rico, BS, Cuyahoga County Medical Examiner's Office, 11001 Cedar Avenue, Cleveland, OH 44106; Claire Kaspar-Naso, BS, Cuyahoga County MEO, 11001 Cedar Avenue, Cleveland, OH 44106; Harold E. Schueler, PhD, Cuyahoga County Medical Examiner's Office, 11001 Cedar Avenue, Cleveland, OH 44106; Paula Wallace, BA, Cuyahoga County Medical Examiner's Office, 11001 Cedar Avenue, Cleveland, OH 44106; and Thomas P. Gilson, MD, Cuyahoga County MEO, 11001 Cedar Avenue, Cleveland, OH 44106*

After attending this presentation, attendees will better understand the frequency and demographics of PCP-related cases seen at the Cuyahoga County Medical Examiner's Office (CCMEO) in Cleveland, OH, from 2006 to 2014.

This presentation will impact the forensic science community by informing forensic professionals about a subset of PCP-positive DUID cases and homicides characterized by PCP use prior to death in the City of Cleveland.

Originally developed as a surgical anesthetic in the 1950s, PCP (1-(1-phenycyclohexyl) piperidine) was effective due to its ability to enter patients into trance-like or "dissociative" states; however, due to negative side effects, its use as an anesthetic was discontinued. Today, PCP is a Schedule II drug that causes behavioral responses ranging from hallucinations to disorientation, severe manic states, increased pain threshold, and overall mimicking symptoms of schizophrenia. Research has linked PCP use with generally violent and aggressive behavior including self-injury, aggressiveness toward others, and lack of driving competency. While the PCP-abuse decades of the 1980s and 1990s have waned, Drug Abuse Warning Network (DAWN) data indicates a 400% increase of PCP-related emergency room visits between 2005 and 2011. In this study, the incidence of PCP in Cuyahoga County in both antemortem DUID cases and postmortem cases seen at CCMEO are evaluated.

The cases in which PCP was positively identified by screening blood, urine, or other biological matrices by Enzyme-Multiplied Immunoassay Technique (EMIT), **Enzyme-Linked Immuno-Sorbent Assay (**ELISA), or gas chromatography (with Nitrogen–Phosphorus Detector (NPD)) and confirmed by Gas Chromatography/Mass Spectrometry (GC/MS) between 2006 and 2014, were gathered and analyzed by means of a statistical package included in CCMEO's Pathways© program. Testing sensitivity levels were consistent across testing methodologies during the period. Antemortem samples were submitted by local police departments for cases of DUID in which the individual was stopped for erratic driving. Postmortem cases involving PCP-positive toxicology results were assessed for cause of death, decedent demographics, location of death, and polysubstance abuse.

Out of the total PCP-positive cases for the nine-year period, 68.50% were DUID cases and 31.50% were postmortem cases. Specifically, the incidence of PCP-positive DUID cases has increased five-fold over the past nine years with 1.04% PCP-positive cases in 2006 compared to 5.42% for 2014. Within these DUID cases, the blood PCP concentration range was 0.01mg/L -0.18mg/L with a median value of 0.05mg/L. Polysubstance abuse occurred in 85.4% of the DUID cases. The most co-consumed drugs of interest were cannabinoids (THC), present in 40.00% of the DUID cases, followed by ethanol in 21.95% of the cases, and cocaine in 12.68% the cases.

Postmortem cases positive for PCP were analyzed for cause of death. Homicides made up 47.19% of the total postmortem PCP cases, followed by other causes of death such as overdoses from acute intoxication of PCP or from other drugs such as heroin in 17.98% of these cases and suicides in 12.36% of these cases. The most significant finding was the large number of homicides involving PCP-positive decedents. Relative to the total homicide cases seen at CCMEO, on average, PCP positive homicides make up 3.31%, with a maximum of 6.99% observed in 2012. Within the PCP-positive homicides, 80.48% involved Black males, 7.14% involved Black females, and 2.38% involved White males. Of the PCP-positive homicides, 85.71% were single with a median age of 31 years old; 86.49% of these homicides occurred within the City of Cleveland and the other 13.51% occurred in the suburbs. The blood PCP concentration range was 0.05mg/L 0.5mg/L with a median value of 0.16mg/L. Further toxicological analysis shows that 73.1% of the cases also tested positive for other drugs of interest. Ethanol was present in 53.66% of the homicide cases, followed by THC at 46.34%, and cocaine at 14.63%.

Post-evaluation of the circumstances of PCP incidence in Cuyahoga County has revealed a subset of homicide decedents that have consumed PCP in addition to other drugs such as marijuana and/or cocaine prior to death. PCP-related DUID cases also rose in this nine-year period, which includes an increase in cases from the Cleveland Police Department starting in 2009. While the relationship of PCP use and aggressive or reckless behavior is not well defined, this epidemiological study shows the association between PCP usage and violent crimes such as homicide and dangerous driving. Perhaps this data can shed light on PCP usage in Cuyahoga County, raising awareness about the association between PCP use, homicide incidence, and DUIDs.

Phencyclidine, Homicides, DUID

K4 Patterns of Drugs and Poisons on Criminal Cases in Southeastern Korea (Busan, Ulsan, and Gyeongsangnam-Do) for 2014

Eunmi Kim, PhD, Busan Institute, National Forensic Service, 50 Geumoh-ro Mulgeum-eup Gyeongsangnam-do, Yangsan, SOUTH KOREA; Hongil Ha, Busan Institute, National Forensic Service, 50 Geumoh-ro, Mulgeum-eup, Yangsan 626-742, SOUTH KOREA; Park Yonghoon, 50 Geumoh-ro Mulgeumeup, Yangsan-City, Gyeongnam 626-815, SOUTH KOREA; and Hee-Sun Chung, PhD, Graduate School of Analytical Science and Tech, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 305-764, SOUTH KOREA*

After attending this presentation, attendees will better understand the recent patterns of drugs and poisons on criminal cases in the southeastern area of Korea.

This presentation will impact the forensic science community by providing the statistics of drugs and poisons in the southeastern area of Korea and by helping attendees understand the current state of forensic toxicology in South Korea.

The southeastern area of Korea consists of three sectors: Busan, Ulsan, and Gyeongsangnam-do. It is a relatively small area in South Korea in both population (eight million) and land area (12,344 km^2), comprising 16% and 12% of South Korea, respectively. Busan institute of the National Forensic Service (NFS) investigated approximately 50,000 cases throughout the southeastern area in 2014, more than 20% of the total cases covered by NFS. In this study, patterns of drugs and poisons on criminal cases in the southeastern area of Korea were investigated. The investigation was conducted by iLIMS, the laboratory information management system of NFS, between January and December of 2014. The objectives were as follows: (1) the fatal cases of drug or poison intoxication from the autopsy were investigated; (2) the types and frequencies of detected drugs in postmortem blood were examined; and, (3) case numbers by sample type and detection (positive) rates of illicit drugs in specimens from drug abusers were investigated. In addition, Novel Psychotropic Substances (NPSs) detected in seized materials in 2014 were also described.

A total of 606 autopsy cases were conducted by the Busan institute of the NFS in 2014. Among them, 15 cases determined drug or poison intoxications as the Cause Of Death (COD), making up 2.5% of the total cases. Hypnotic sedatives such as zolpidem, diazepam, clomipramine, alprazolam, and the illicit drug methamphetamine were detected. Additionally, pesticides such as nereistoxin, cyhalothrin, indoxacarb, glyphosate, glufosinate, and bifenthrin were detected. A surfactant and acetic acid were also detected in the postmortem specimens.

A total of 108 drugs in postmortem blood were detected from the autopsy cases, and the top five most frequently detected drugs were chlorpheniramine (n=46), tramadol (n=36), diazepam (n=29), zolpidem (n=25), and lidocaine (n=24). These drugs were supposed to have been used for therapeutic purposes in the hospital rather than for suicidal or homicidal purposes.

Meanwhile, a total of 1,728 cases were submitted for illicit drug testing in 2014. Among them, hair was the most common type of submitted specimen (787 cases), making up 46% of the total, followed by urine (37%) and seized material (18%). The detection rates (positive rates) of methamphetamine in hair, urine, and seized material were 62.9%, 51.2%, and 56.6%, respectively. This indicates that methamphetamine is still the most abused drug in Korea. In addition, the abuse of NPSs has been increasing dramatically since the late 2000s worldwide (Chung et al).[1] A total of ten different NPSs were identified in seized materials in 2014. Among them, eight were synthetic cannabinoids and two were alkylnitrites. The detected compounds were JWH-018, JWH-073, 5F-UR-144 (XLR-11), QUPIC (PB-22), XLR-12, 5F-AMB, 5F-MN-18, EAM-2201, NM-2201, 5F-ADB-PINACA, isobutylnitrite, and isopentylnitrite.

Even though the southeastern area has a relatively small area and population in South Korea, Busan institute is ranked in second place among NFS institutes in terms of case numbers, following Seoul institute. It is useful to understand the patterns of drugs and poisons in the southeastern area, in terms of how the results reflect the current state of South Korea in forensic toxicology.

Reference(s):

1. Chung H., Lee J., Kim E. Trends of novel psychoactive substances (NPSs) and their fatal cases, *Forensic Tox*, DOI 10.1007/s11419-015-0286-5, Aug 2015.

Southeastern Korea, Drugs, Poisons

K5 Case Report: Detection of 25C-NBOMe in Three Related Cases

John J. Kristofic, BS, Armed Forces Medical Examiner System, 115 Purple Heart Drive, Dover AFB, DE 19902; Jeffrey D. Chmiel, MS, Armed Forces Medical Examiner System, 115 Purple Heart Drive, Dover AFB, DE 19902; George F. Jackson, PhD, Armed Forces Medical Examiner System, 115 Purple Heart Drive, Dover AFB, DE 19902; Erin Karschner, PhD, 603 Friendship Village Drive, Harrington, DE 19952; Eric T. Shimomura, PhD, Armed Forces Medical Examiner System, 115 Purple Heart Drive, Dover AFB, DE 19902; Shawn P. Vorce, BS, Armed Forces Medical Examiner System, 115 Purple Heart Drive, Dover AFB, DE 19902; Justin Holler, MS, Armed Forces Medical Examiner System, 115 Purple Heart Drive, Dover AFB, DE 19902; Stephen L. Robinson, MD, 115 Purple Heart Drive, Dover, DE 19902; and Thomas Z. Bosy, PhD, Armed Forces Medical Examiner System, 115 Purple Heart Drive, Dover AFB, DE 19902*

After attending this presentation, attendees will be familiar with three related cases involving 25C-NBOMe, including a fatal intoxication.

This presentation will impact the forensic science community by reporting a complete tissue distribution of 25C-NBOMe in a postmortem case with antemortem findings in two related cases.

A 23-year-old male became agitated and combative shortly after being detained by police. The individual continued to resist while he was handcuffed and placed prone on the ground. He was subdued by an electronic control device and oleoresin capsicum spray. While prone, the individual experienced respiratory problems and life-saving measures were initiated. He was transported to the hospital and pronounced dead. An autopsy was performed and tissues submitted for toxicological analysis. As part of the investigation, blood and urine specimens from two individuals associated with the decedent were also submitted for analysis.

Specimens from all cases were subjected to volatile analysis by headspace/gas chromatography equipped with a flame ionization detector and immunoassay analysis (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, opiates, oxycodone, phencyclidine, 6-acetylmorphine, and lysergic acid diethylamide). Postmortem specimens were screened for alkaline-extractable drugs by Gas Chromatography/Mass Spectrometry (GC/MS). Based on case history, an additional urine screen was performed on all three cases by Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS). 25C-NBOMe and proposed metabolites were detected in each case. 25C-NBOMe results were confirmed and quantitated using Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS). A standard addition protocol was implemented for all tissue aliquots to account for matrix effects.

	Matrix	25C-NBOMe ng/mL or ng/g)	2C- (ng/mL or ng/g)
Case #1	Blood (Heart)	2.07	0.12
(Postmortem)	Urine	27.43	0.38
	Vitreous	0.50	-
	Adipose	2.35	-
	Brain	19.10	-
	Spleen	27.13	-
	Lung	25.21	-
	Liver	15.20	-
	Kidney	25.06	-
	Gastric	302.41	-
Case #2	Blood	0.48	-
	Urine	1.73	0.20
Case #3	Blood	1.04	-
	Urine	4.06	0.11

Full-scan GC/MS alkaline drug screen detected pseudoephedrine, nicotine, and cotinine in the urine of Case #1. Pseudoephedrine was confirmed in urine by GC/MS, but was not detected in the decedent's blood at a 50ng/mL cutoff.

The cause of death was ruled 25C-NBOMe toxicity temporally associated with excited delirium and forcible restraint. The manner of death was ruled accidental.

The opinions or assertions presented herein are the private views of the authors and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the United States Army Medical Research and Materiel Command, or the Armed Forces Medical Examiner System

25C-NBOMe, Postmortem, Tissue Distribution

K6 Determination of Synthetic Hallucinogens: 25I-, 25C-, and 25B-NBOMe by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Using D3-25I-NBOMe Internal Standard

Joseph A. Cox, MS, 3520 Burke Road, Pasadena, TX 77504; Naga Venkata Naidu, PhD, Expertox, 1430 Center Street, Ste C, Deer Park, TX 77536; and Ernest D. Lykissa, PhD, Expertox, 1430 Center Street, Ste C, Deer Park, TX 77356*

After attending this presentation, attendees will better understand a sensitive method for determining concentrations of 25I-, 25C-, and 25B-NBOMe in toxicology specimens using LC/MS/MS.

This presentation will impact the forensic science community by relaying a novel procedure that utilizes the sensitivity and selectivity of LC/MS/MS, while promoting lower Limit Of Detection (LOD) than previous studies. In addition, using a deuterated NBOMe (D3-25I-NBOMe) makes this method more reliable and robust in determining NBOMe drugs in blood and urine.

Among drug-using populations, use of designer compounds has increased over the past decade. An emerging synthetic LSD compound (NBOMe) has joined the popular designer compounds such as cathinones (bath salts) and synthetic cannabinoids (e.g., JWH). In postmortem cases, the average concentrations of NBOMe drugs encountered were less than 0.5ng/mL in biological specimens. Lower concentrations increase the need for a sensitive method to determine NBOMe drugs in biological samples. NBOMe drugs are available as powders, liquid solutions, laced on edible items, and soaked into blotter paper. In November 2013, the United States Drug Enforcement Administration placed three NBOMe drugs (25I-, 25C-, and 25B-NBOMe) on the Schedule I list for two years citing lack of medical use or human consumption. Street names for NBOMe drugs include: N-Bomb, solaris, cimbi-5, synthetic LSD, and Smiles. The NBOMe class drugs are extremely potent 5-HT2A agonists, particularly 25I-NBOMe.

Specifically, 1mL aliquots of blood and urine samples fortified with varying concentrations of the three NBOMe drugs and D3-25I-NBOMe as an Internal Standard (IS) were extracted with an organic solvent under basic conditions. Calibrators were at concentrations of 5pg/mL, 10pg/mL, 20pg/mL, 50pg/mL, 100pg/mL, and 500pg/mL. Chromatographic separation was achieved on a C-18 column with gradient elution. Mobile phases of water:methanol (90:10v/v) with 5mM ammonium formate (solvent A) and acetonitrile with 0.1% formic acid (solvent B) were used in a gradient elution program; 30% to 70% B over in 3mins, returning to initial 30% of B over in 0.5mins, and held for 0.5min for a total run time of 4min. Data was acquired on positive mode, Multiple Reaction Monitoring (MRM) transitions monitored for 25I (428m/z-121/91m/z), 25B (382m/z–121/91m/z), and 25C (336mz–121/91m/z).

The calibration range of this method was shown to be linear (R^2=>0.99) from 5pg/mL to 500pg/mL for all three NBOMe drugs in blood and urine with a LOD and Limit of Quantitation (LOQ) of 5pg/mL. The R^2 values for 25I-, 25B-, and 25C-NBOMe in urine were 0.9996, 0.9999, and 0.9997, and in blood were 0.9967, 0.9987, and 0.9997, respectively. The precision (%CV) at the LOQ for 25I-, 25B-, and 25C-NBOMe in urine were 12.6, 17.3, and 2.2, and in blood were 6.2, 7.0, and 4.6, respectively.

The method was validated and the calibration curves reconcile well with forensic toxicology criteria. The extraction and LC/MS/MS method developed for analysis of blood and urine for 25I-, 25B-, and 25C-NBOMe is precise, sensitive, and reproducible at forensically relevant concentrations.

25I-NBOMe, Synthetic LSD, D3-25I-NBOMe

K7 The Rapid Identification of Synthetic Hallucinogens 25I-NBOMe and 2C-B Using DART®-MS

Joseph Stone, BS, 101 N 5th Street, Apt 615, Richmond, VA 23219-0010; Justin L. Poklis, BS, Virginia Commonwealth University, Dept of Pharmacology & Toxicology, 410 N 12th Street, Rm 746, PO Box 980613, Richmond, VA 23219-0613; Michelle R. Peace, PhD, VA Commonwealth University, 1015 Floyd Avenue, PO Box 843079, Richmond, VA 23284; and Alphonse Poklis, PhD, Virginia Commonwealth University, Dept of Pathology-Toxicology Laboratory, Box 98-165 MCVH/VCU Station, Richmond, VA 23298-0165*

After attending this presentation, attendees will be able to utilize the method presented in order to rapidly identify synthetic hallucinogens.

This presentation will impact the forensic science community by providing a quick screening method using direct analysis in real time AccuTOF™ Mass Spectrometry (DART®-MS) for the analysis of synthetic hallucinogens. This presentation provides a means to identify these compounds found on blotter paper and as a powder.

Synthetic hallucinogens, such as Alexander Shulgin's series of ring substituted methoxyphenethylamines (2-C) and the relatively new dimethoxyphenyl-N-[(2-methoxyphenyl) methyl]-ethanamine (NBOMe) derivatives, have recently become available via the internet. These designer drugs are potent serotonin 5-HT_{2A} receptor agonists. This receptor is linked to certain cognitive processes and other complex behaviors, including working memory, and is responsible for the hallucinogenic effects caused by drugs such as Lysergic Acid Diethylamide (LSD). Numerous 2-C and NBOMe derivatives are available. These drugs are currently sold as "research chemicals" in powder form or on blotter paper. This study presents two cases of the rapid identification by DART®-MS of NBOMe and 2-C derivatives on blotter paper and a "research chemical" in an unmarked capsule, respectively.

Methods: The blotter paper was analyzed by the DART®-MS as both the blotter paper and as a methanol extract. The white powder sample was analyzed directly using the DART®-MS. The DART®-MS was operated in positive-ion mode and controlled by Mass Center software version 1.3.4 m. The ion source had the helium gas flow rate at 2.0L/min, gas heater temperature of 300°C, discharge electrode needle at 4,000V, electrode 1 was set at 150V, and electrode 2 at 250V. The resolving power of the mass spectrometer was 6,000 Full Width at Half Maximum (FWHM). Measurements were taken with the ion guide peak voltage of 800V, reflectron voltage of 900V, orifice 1 was operated at 300°C in function switching mode (20V, 60V, and 90V) with orifice 2 set at 5V and the ring lens set at 3V. The measured mass range was from 40Da to 1,100Da. Accuracy of the data was evaluated by a mass difference of ±5mmu. Polyethylene glycol 600 served as primary reference material for exact mass measurements in each data acquisition set. The 20V DART®-MS spectra of the samples were used to identify the NBOMe and 2-C derivatives.

Results: The blotter paper was determined to contain both 25I-NBOMe and N-(2-methoxybenzyl)-2, 5-dimethoxy-4-chlorophenethylamine (25C-NBOMe) as the major components and smaller amounts of NBOMe derivatives including 2-(4-chloro-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl] ethanamine (25C-NBOMe), 2-(2, 5-dimethoxyphenyl)-N-(2-methoxybenzylidene) ethanamine (25H-NBOMe), 25I-MBOMe-Imine, and 25H-NBOMe-Imine. The "research chemical" was determined to contain 4-bromo-2, 5-dimethoxyphenethylamine (2C-B).

Conclusion: Given the efficacy and ease of synthesis of these types of synthetic hallucinogens, it is possible that the abuse of these drugs will continue. DART®-MS was found to be a rapid screening method for the accurate identification of synthetic hallucinogens on both blotter paper and in powder form with little to no sample preparation.

This project was supported in part by the National Institute of Health (NIH) Center for Drug Abuse.

DART®-MS, 25I-NBOME, 2C-B

K8 Fragmentation Pathways and Structural Characterization of Synthetic Cathinones Using Electrospray Ionization (ESI) and High Resolution Mass Spectrometry

Lindsay Glicksberg, 2451 Lake Road, #511, Huntsville, TX 77340; Kelsie Bryand, MS, PO Box 2525, Huntsville, TX 77340; and Sarah Kerrigan, PhD, Sam Houston State University, 1003 Bowers Boulevard, SHSU Box 2525, Huntsville, TX 77341*

After attending this presentation, attendees will understand the importance of structural identification of fragment ions and be able to identify the common fragmentation pathways of synthetic cathinones.

This presentation will impact the forensic science community by increasing fundamental understanding of fragmentation pathways associated with the cathinone designer drugs.

Designer drugs continue to present a number of challenges to the forensic toxicology community. Synthetic cathinones are a growing class of psychostimulants that can be chemically characterized as beta-keto amphetamines. Although derived from cathinone (*Catha edulis*), these synthetic drugs are substituted at the phenyl ring, amino group, or propoanone terminus. The functional substituents greatly influence fragmentation pathways and ion formation in both Electron Impact (EI) and ESI. In addition to their low boiling points and volatility in the base (uncharged) form, several of the cathinones are thermally labile and degrade during Gas Chromatographic (GC) analysis. Many of the cathinones, particularly the tertiary amines (pyrrolidine derivatives), undergo extensive fragmentation in EI, yielding poorly specific mass spectra with a limited number of diagnostic ions for selected ion monitoring. Liquid Chromatography/Mass Spectrometry (LC/MS)is advantageous from the standpoint of increased thermal stability and the ability to optimize the conditions during ionization to yield highly specific fragment ions using ESI. Moreover, high resolution mass spectrometry is a powerful tool for structural elucidation. This presentation describes the fragmentation pathways and structural characterization of synthetic cathinones using LC/quadrupole Time Of Flight/MS (LC/qTOF/MS).

A validated method for the determination of 22 synthetic cathinones in urine using LC/qTOF/MS has been previously reported. A total of nine isotopically labeled internal standards were used. The principal compounds of interest were: methcathinone; 3-Fluoromethcathinone (3-FMC); 4-Fluoromethcathinone (4-FMC); ethcathinone; ethylone;, methedrone;, buphedrone; butylone; mephedrone; eutylone; 4-Methylethcathinone (4-MEC) ; 3,4-Methylenedioxy-α-Pyrrolidinobutyrophenone (MDPBP); pentedrone; pentylone; 3,4-Dimethylmethcathinone (3,4-DMMC) ; α-Pyrrolidinopentiophenone (α-PVP); 4-Ethylmethcathinone (4-EMC); 4-Methyl-α-Pyrrolidinobutiophenone (MPBP); Methylenedioxypyrovalerone (MDPV); pyrovalerone; and naphyrone. The target compounds include a variety of secondary and tertiary amines, methylenedioxy derivatives, benzylic, and amino substituents. Following optimization of ionization conditions, fragmentation pathways were investigated.

In addition to the formation of stable immonium ions (that predominate EI spectra), other common fragmentation pathways involved neutral losses of water, amines and CH_4O_2. Although non-specific neutral losses (such as water) should be avoided during targeted analyses, formation of phenyloxazole and alkyldioxybenzoyloxonium cations arising from the loss of CH_4O_2 and amines in methylenedioxy derivatives provide improved structural specificity. Synthetic cathinones bearing a tertiary amine are also characterized by stable ions arising from the loss of pyrrolidine. In contrast to the secondary amines, water losses are not observed for the pyrrolidinyl derivatives due to the limited mobility of the hydrogen on the amino group. Many of the ring substituted and non-ring substituted secondary amine synthetic cathinones resulted in the formation of radical cations. Loss of the ketone with subsequent rearrangement to form a cyclic cation was prevalent among the secondary amines.

Tandem Mass Spectrometry (MS/MS) spectra arising from collision-induced dissociation should be evaluated carefully to enhance method specificity, as well as sensitivity. The selection of ions based on abundance alone is strongly discouraged. Structural characterization of fragments and optimization of their abundance during method development is important in terms of specificity and overall robustness of the method. Not only does this approach improve overall analytical performance, but these characteristic losses can also provide useful information for the identification of emerging or as-yet unidentified cathinone analogs and derivatives.

Synthetic Cathinones, Fragmentation, LC/qTOF

K9 Development and Validation of a Confirmatory Method for Six Novel Psychoactive Substances (NPS) in Whole Blood Using Ultra Performance Liquid Chromatography/Tandem Mass Spectrometry (UPLC/MS/MS)

Melissa Friscia, MSFS, 429 Grand Avenue, Langhorne, PA 19047; Amanda L.A. Mohr, MSFS, Center for Forensic Science, Research & Education, 2300 Stratford Avenue, Willow Grove, PA 19090; Francis X. Diamond, BS, 3701 Welsh Road, Willow Grove, PA 19090; and Barry K. Logan, PhD, NMS Labs/CFSRE, 3701 Welsh Road, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to implement a method for the confirmation of six prevalent NPS in whole blood including methylone, dimethylone, ethylone, butylone, 4-Fluoroamphetamine (4-FA), and alpha-Pyrrolidinopentiophenone (alpha-PVP).

This presentation will impact the forensic science community by providing evidence of a robust and reliable analytical method capable of quantifying the target compounds and meeting the requirements for method validation established by the Scientific Working Group for Forensic Toxicology (SWGTOX). The method focuses on novel compounds with which forensic laboratories have relatively limited experience in routine casework.

The use of NPS within the United States has recently been a focus of media attention due to drug-related deaths, emergency room visits, and large-scale hospitalizations or medical aid calls. Due to the increase in use, and a lack of resources for the analysis of some of the most currently abused compounds, there is a significant need to develop toxicological procedures for the measurement of these analytes in forensic specimens. The assays can then be used to study the prevalence of NPS use in various populations and the investigation of specific forensic cases. In addition, given the proliferation of NPS drugs in the United States marketplace with very similar structures and identical molecular formula, there is an urgent need for methods that can distinguish between closely related compounds, isomers, and isobars.

The increasing popularity of NPS and recreational research chemicals is easily documented by monitoring online forums and discussion groups of Electronic Dance Music (EDM) festival attendees and other groups associated with EDM culture. This method was developed and validated for the confirmation of the most prominent drugs identified from screening samples collected at an EDM event. The purpose of this project was to develop a method using UPLC/MS/MS for the confirmation of six NPS including methylone, dimethylone, ethylone, butylone, 4-FA, and alpha-PVP, which had screened positive in biological specimens (blood, urine, and oral fluid) collected from attendees at an EDM festival.

Samples (0.5mL) were prepared for analysis using a basic liquid-liquid extraction with 0.1M borate buffer (pH=10.4) and n-butyl chloride:ethyl acetate (70:30). The organic phase was evaporated to dryness and the samples were reconstituted in 90:10 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B). Chromatographic separation was achieved on a UPLC/MS/MS system (Waters® Acquity UPLC coupled with a Waters® Quattro Micro API mass spectrometer) using positive electrospray ionization and multiple reaction monitoring mode with an Acquity UPLC® BEH C18 (50mm x 2.1mm, 1.7µm) at 50ºC. Mobile phase was introduced into the system in a gradient programmed with 15% B, isocratic for one minute, that was linearly increased to 35%B over five minutes, increased again to 90% B over one minute before the gradient was returned to the initial conditions and held for 2.9 minutes. The flow was set to 0.2mL/min with a total run time of eight minutes.

The method was developed to be a highly sensitive assay, with optimal run time. The method was validated following guidelines set forth by the SWGTOX. The method was linear between 5ng/mL and 500ng/mL and had a defined limit of quantitation and detection of 5ng/mL for all analytes. Recovery for all analytes was greater than 85%. The method was free from carryover at five and ten times the highest calibrator, from interferences from matrix effects, and from interferences from commonly encountered and related analytes at various levels (n=20). The within-run precision ranged from 3.2% to 5.6% for the low control at 15ng/mL and 2.0% to 4.2% for the high control at 350ng/mL. For the low and high controls respectively, the between run precision ranged from 4.4% to 7.7%, and 2.6% to 6.5%, and the accuracy ranged from -3.2% to 3.7% and -0.8% to -5.8%. The method was matrix matched to urine and was found to be acceptable, producing a between-run precision of 1.8% to 12.5%, and 2.2% to 8.3% for the low and high controls, respectively.

The method was applied for the confirmation and quantitation of the target drugs in blood samples from 17 human subjects whose samples had screened positive for these substances.

NPS, UPLC/MS/MS, Cathinones

K10 The Application of Gold Nanoparticles for the Trace Detection of PINACAs in Urine by Surface Enhanced Raman Spectroscopy (SERS)

Thaddeus Mostowtt, MFS, 16020 S Post Road, Apt 204, Weston, FL 33331; and Bruce R. McCord, PhD, Florida International University, Dept of Chemistry, University Park, Miami, FL 33199*

After attending this presentation, attendees will understand the principles of SERS, how SERS can be used to create a low limit of detection for synthetic cannabinoids, the effect of using different aggregating agents when combined with gold nanoparticles to enhance the limit of detection, and how SERS can be a fast and easy analysis for drug detection in toxicological samples.

This presentation will impact the forensic science community by demonstrating the application of SERS as a useful procedure of detecting trace levels of synthetic cannabinoids in solution that is rapid, sensitive, and applicable to a variety of biological matrices.

The use and abuse of synthetic cannabinoids has increased significantly in recent years due to their easy access and growing popularity in young adults. Initially, these drugs, known as "Spice" or "K2," were sold in retail outlets or via the internet and labeled as "not for human consumption" to avoid any possible regulation of the products by the Food and Drug Administration. This popularity has led to an increase in emergency room visits due to synthetic cannabinoid intoxication in recent years. As more of these drugs become illegal, new synthetic legal versions of these drugs are being made, which presents problems for the forensic scientist as standard methods may not detect the target drug.

The most common method of screening detection for drugs of abuse in biological samples is the immunoassay; however, this method presents some disadvantages, particularly for newly synthesized compounds which may not respond to the test. Other problems include cross-reactivity between different synthetic cannabinoids, hook effects, and high cut-off values for determining if the drug is present. More advanced methods have also been used, such as Gas Chromatography/Mass Spectrometry (GC/MS); however, these procedures involve complex sample preparation and long run times. A potential solution to this issue is SERS.

Raman spectroscopy is an under-utilized technique for the detection and identification of drugs due to its perceived low sensitivity for analytes in solution using traditional procedures; however, when Raman spectroscopy is performed in the presence of metallic nanoparticles, signal can be enhanced several orders of magnitude, and this is known as SERS. The addition of aggregating agents, generally ionic salts, further increases the signal via the creation of hot-spots due to displacement of the stabilizing agent which leads to a change in the surface of the metallic nanoparticle and the ionic strength of the solution. This method has already been confirmed to work for the toxicological detection of benzodiazepines with limits of detection ranging from 1ng/mL to 200ng/mL.

In this project, gold nanoparticles were prepared using a sodium citrate reduction and aggregating agents were used to enhance the Raman signal of four different synthetic cannabinoids: APINACA (AKB48), AB-PINACA, ADB-PINACA, and AB-CHMINACA, and their metabolites. Seven different aggregating agents including MgCl2, CaCl2, KCl, NaCl, MgSO4, KNO3, and Na2SO4 were examined at varying concentrations to optimize sensitivity of detection. Once optimized, the SERS method was used to analyze spiked urine samples containing the parent drug and metabolites. Other factors, including the concentration of nanoparticles, time, and temperature, were also examined. Upon analysis, the Raman spectrum of each synthetic cannabinoid could be easily distinguished and mixture resolution was possible using the KnowItAll® computer software program.

These results demonstrate that SERS can be utilized to detect trace amounts of PINACAs and their metabolites in aqueous solutions. Therefore, following rapid extraction of the analyte, SERS can be used as a detection method of synthetic cannabinoids in urine and saliva samples, which can be useful in forensic toxicology laboratories.

SERS, PINACAs, Toxicology

K11 Forensic Medical Evaluation of Fatalities Resulting From Lighter Gas Inhalation

Erdinc Ozdemir, The Council of Forensic Medicine, Mus Courthouse, Forensic Medicine, Mus, TURKEY; Ibrahim Üzün, Council of Forensic Medicine, Istanbul, TURKEY; Muhammet Demir, Istanbul Adli Tip Kurumu, Istanbul, TURKEY; and Huseyin Es, Department of Forensic Medicine, Bingol, TURKEY*

After attending this presentation, attendees will have insight regarding the effects of lighter gas inhalation-related intoxications, forms of utilization, and prevention.

This presentation will impact the forensic science community by illustrating the roles of the forensic toxicology and forensic pathology experts in cases of inhalation intoxication by lighter gas.

Voluntary inhalation/abuse of volatile substances is an important public health problem which especially affects adolescent and young populations worldwide and may be encountered in all socioeconomic and cultural levels. Volatile substance abuse is seen worldwide and is the second most common after marijuana among young people in the United States. A study from Turkey revealed that 15%-20% of the total population have tried volatile substances and its prevalence for life-long use was reported to be 7%-11%. In Turkey, 8.8% of the population have tried volatile substances at least once. Lighter gas abuse-related death is still an important health problem in Turkey. Although some studies on the harmful effects of lighter gas abuse on human health have been conducted in developed countries, detailed epidemiological data on the use of lighter gas abuse is not available in Turkey, although it is known that this problem is gradually becoming widespread.

Volatile substance inhalation is responsible for considerable morbidity and mortality. Sudden death is the most common cause of volatile substance-related deaths. The mechanism of sudden death in volatile substance abuse is clear and includes cardiac arrhythmia, hypoxia, and respiratory depression. In addition to anaesthetic and narcotic effects of n-Butane and isobutane on the central nervous system, its concentration of 0.5%-15% in the air may cause fatal arrhythmia. Reports suggest that n-butane and isobutane increase the level of myocardial catecholamines, and resultant alarm or hard muscle activity/exercise (e.g., running or fright) may accelerate death. Propane is less toxic than n-butane and isobutane. Its cardiac effects are negligible. The Lethal Dose, 50% (LD50) value of propane is higher when compared to n-butane, which makes it less lethal.

Lighter gas abusers directly consume the gas they voluntarily obtain by releasing the gas into a plastic bag in order to increase the effects of the substance. The reason for this is to prolong the duration of inhalation and increase the concentration of the substance. In this way, sudden death may occur due to the tendency of acute asphyxia.

In this study, cause-of-death reports written in the First Forensic Medicine Specialization Board of Forensic Medicine Institution Presidency between 2012 and 2014 were retrospectively analyzed. A cause of death of lighter gas inhalation was found in 37 cases. Biological samples were collected during the autopsy and stored at +4°C for further toxicological analysis. Toxicological analyses were conducted in the forensic toxicology laboratory on various biological samples (blood, urine, bile, vitreous humor, gastric content, brain, lung, liver, and kidney) sent from either the mortuary department where the medicolegal autopsies were performed or from other forensic facilities across the country. Headspace/Gas Chromatography/Mass Spectrometry (HS/GC/MS) was used during analyses. The gas-tight vials were stored at -20°C for further toxicological examination, if needed. All subjects were male with a mean age of 16.3 years. It was determined that 24.3% of the cases used a plastic bag to increase the effects of lighter gas and 75.7% inhaled the lighter gas via their mouth and nose. In 91.8% of the cases, crime scene investigation teams found lighter gas tubes, while no evidence of lighter gas use was found in 8.2% of the cases. Toxicological analysis revealed no lighter gas active ingredients (n-butane, isobutane, propane) in 62.2% of the cases, while n-butane and n-butane+propane were detected in 35.1% and 2.7% of the cases, respectively.

The importance of lighter gas inhalation-related deaths in Turkey has been increasing. Strict measures against the abuse of these very dangerous substances should be undertaken by the mutual efforts of medical specialists and legislators.

Volatile Substance, Sudden Death, Poisoning

K12 Domino Effect: A Singular Case of Six Fatal Hydrogen Sulfide (H_2S) Poisonings in Quick Succession — Evaluation of the Sulfides Quantification Method

Nunziata Barbera, MD, University of Catania, Via S Sofia 87, Catania 95123, ITALY; Angelo Montana, MD, University of Catania, Via Santa Sofia, Catania, ITALY; Francesca Indorato, MD, University of Catania, Via S Sofia 87, Catania, ITALY; Nadia Arbouche, SB, University of Catania, Via S Sofia 87, Catania 95123, ITALY; and Guido Romano, SB, University of Catania, Via S Sofia 87, Catania 95123, ITALY*

After attending this presentation, attendees will understand that it is not possible on the basis of toxicological data alone to distinguish between the H_2S concentration in blood secondary to sulphide lethal poisoning and the H_2S produced during putrefaction.

This presentation will impact the forensic science community by describing the importance of circumstantial evidence along with histopathological findings (greenish-blue color of the hypostasis and of the internal organs, of the brain in particular, related to postmortem formation of sulfhemoglobin) in identifying H_2S lethal poisoning along the pathological pathway leading to death.

H_2S is one of the major toxic gases in forensic practices; it is a colorless gas and has a strong odor of rotten eggs.[1] Aside from being a by-product of many industrial processes, this gas is naturally produced during the putrefaction of organic substances.[2,3] H_2S is absorbed by the upper respiratory tract mucosa and causes histotoxic hypoxemia and respiratory depression by exerting an inhibitory effect on cytochrome oxidase.[4]

This study reports six autopsy cases of fatal H_2S poisonings due to the inhalation of H_2S gas because of an occupational accident. In fact, the six men died during the unblocking of a wastewater cistern. The first worker died shortly after clearing the occlusion, the other five died one by one in an attempt to help their colleagues.

The goal of this study was to evaluate the role of toxicological data with the possibility of distinguishing between the H_2S concentration in blood secondary to lethal poisoning and the endogenous H_2S produced during putrefaction. For this purpose, the postmortem H_2S concentrations of the six men who died at work were compared with the postmortem concentrations of endogenous H_2S in blood samples from 12 subjects who died from different causes. At autopsy, femoral blood samples were collected at different Postmortem Intervals (PMIs) — the 12 subjects were divided according to the PMIs into three groups (first group: 24-36 hours; second group: 37-72 hours; third group: 73-120 hours).

Toxicological analysis: 0.2ml of blood sample was added to a mixture of 0.5ml of 20mM PFBBr solution in toluene, 2.0ml of internal standard solution (10mM of TBB in ethyl acetate), and 0.8ml of 5mM TDMBA solution in oxygen-free water saturated with sodium tetraborate. The preparation was vortexed for 1min and 0.1g of potassium dihydrogenphosphate was added to the mixture. The preparation was again vortexed for 10s and centrifuged at 2,500rpm for 10min. An aliquot of the organic phase was then injected into the Gas Chromatography/Mass Spectrometry (GC/MS) apparatus.

The results of this study showed femoral blood H_2S concentrations (n=6 poisonings) ranged from 8.7mg/L to 28.6mg/L and femoral blood endogenous H_2S concentrations (n=12 deaths from other causes) ranged from 2.2mg/L to 32.7mg/L. A significant relationship between PMI and H_2S concentration was observed. In the 12 blood samples analyzed, sulphide blood concentrations were already detectable at 24 hours after death.

The performed analysis led to the affirmation that it is not possible, based only on toxicological data, to distinguish between the H_2S concentration in blood secondary to sulphide lethal poisoning and the H_2S produced during putrefaction.

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Hydrogen Sulfide, Poisoning, Forensic Toxicology

K13 An Extremely Rare Suicidal Intoxication With Sodium Azide: A Case Report

Francesco Randazzo, University of Pavia, via Forlanini n. 12, Pavia 27100, ITALY; Massimiliano Scida, Via Forlanini 12, Pavia, ITALY; Alessandro De Gaetano, Via Forlanini 12, Pavia, ITALY; Marco Motta, Via Guicciardini, Varese, ITALY; Antonella Profumo, University of Pavia, Department of Chemistry, Pavia, ITALY; Angelo Groppi, via Pietri, 27058, Voghera, Pavia, ITALY; and Luca Morini, via Aselli 52, 27100, Pavia, ITALY*

After attending this presentation, attendees will show the importance of interplay between the on spot investigation performed by the forensic-doctor and the lab work for the identification of the causes of death.

This presentation will impact the forensic science community by the presentation of a rare suicidal intoxication case and also the importance of crime scene investigation in forensic toxicology.

A 23-year-old female doctor in biochemistry was discovered dead on the floor of her home by her work colleagues. A computer displaying web pages regarding suicide and a hand written note that said " …be careful because the poison I used can produce toxic gas in contact with acid in the stomach…" were found. A cup filled with a white liquid and a can containing traces of a white powder were noticed by the forensic-doctor and were collected. The external inspection of the cadaver didn't show any signs of external lesions. A brown material stained the mouth and nose.

At autopsy, the aforementioned brown material was found in the upper and lower airways; the stomach was remarkably stretched by gas and full of beige granular semi-fluid material; and the organs were all congested. Blood, bile, urine, and gastric content samples were collected, as well as lung, kidney, and liver specimens.

All the samples were tested for routine toxicological analysis: Gas Chromatography/Mass Spectrometry (GC/MS), a targeted screening method by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) and Gas Chromatography With Flame Ionization Detector (GC/FID) for alcohol and other volatile substances were all used on the blood and on the gastric content; colorimetric tests for the identification of cyanide and other common poisons were performed on gastric content. Nonetheless all the analytical procedures didn't provide any conclusive results.

Finally, going through the objects retrieved on the scene, it was proposed to analyze the powder inside the can. An Infrared (IR) and an Atomic Absorption Spectroscopy (AAS) analysis were performed, identifying with the former the presence of azide ions (confirmed by High-Performance Liquid Chromatography (HPLC)), and with the latter the presence of sodium. Azide is a highly toxic substance that causes several effects in the body, such as oxidative enzymes inhibition, blockage of oxygen transport and hypotension. A liquid chromatograph analysis using reverse phase (C18) column, isocratic mode, using a mobile phase consisting of a mixture of acetonitrile and water (1:1), with the Diode Array Detector (DAD) operating in the range 200-400nm, was performed on both the powder and the gastric content. The results were confirmed through the method of standard addition on the powder and in the gastric content. The powder was a pure preparation of sodium azide (100%). The gastric content had azide in it, but the concentration could not be evaluated due to the lack of data regarding gastric content weight. An attempt to measure azide was also carried out on the blood, but the results were negative.

Suicide, Sodium Azide, Crime Scene Investigation

K14 Epidemiological and Toxicological Traits in Methadone-Related Deaths — A Five-Year (2010-2014) Retrospective Study in Vojvodina, Serbia

Isidora Samojlik, MD, PhD, University of Novi Sad, Faculty of Medicine, Hajduk Veljkova Street, No 3, Novi Sad 21000, SERBIA; Vesna Mijatovic, MD, PhD, University of Novi Sad, Faculty of Medicine, Dept of Pharmacology, Toxicology, Clinical Pharm, Hajduk Veljkova 3, Novi Sad 21000, SERBIA; Vladimir Knezovic, University of Novi Sad, Faculty of Medicine, Hajduk Veljkova 3, Novi Sad 21000, SERBIA; and Stojan Petkovic, MD, PhD, Faculty of Medicine, University of Novi Sad, Dept of Forensic Medicine, Hajduk Veljkova 5-7, Novi Sad 21000, SERBIA*

After attending this presentation, attendees will be able to characterize and recognize methadone-related deaths among other opioid-related deaths.

This presentation will impact the forensic science community by improving the interpretation of methadone-related deaths along with complex toxicological findings, especially when detected blood concentrations are in therapeutic range (methadone therapeutic concentrations in blood are defined as lower than 1µg/ml, while toxic and lethal concentrations are defined as 1-2µg/ml and >2µg/ml, respectively).

Methadone, a synthetic opioid analgesic, is used in Methadone Maintenance Treatment (MMT) of heroin dependence and chronic pain because of its long half-life, good enteral absorption and low cost; however, in some countries it is being withdrawn from treatment programs because it is becoming an illicit drug of abuse, leading to death following uncontrolled intake. The increasing tendency in Methadone-Related Deaths (MRDs) in the last years is associated with its combination with benzodiazepines and other psychoactive drugs.

Retrospective review of MRDs was conducted at the Institute of Forensic Medicine, Clinical Centre of Vojvodina (Novi Sad, Serbia) serving the population of approximately two million. The study included all relevant cases in a five-year period, from 2010 to 2014. In this study, MRDs were defined as deaths in which methadone was registered in postmortem blood samples, but without other (il)legal opiates or toxic concentrations of other drugs, and where other causes of death were excluded. The collected data were statistically evaluated according to the number of MRDs and Opiate-Related Deaths (ORDs) per year, sex, age, and drugs detected in postmortem samples of peripheral blood. Toxicological screening for alcohol and common drugs was performed by a Gas Chromatography/Mass Spectrometry (GC/MS) method.

During observed five-year period, the absolute number of ORDs in the region of Vojvodina showed an increased frequency with the maximum of 26 ORDs observed in 2010. The proportion of MRDs in those deaths was variable (35-77%) and the total number of MRDs was 54. Among all MRDs cases, methadone was detected in biological samples of six women and 48 men, aged 20 to 59 (median 33±8 years).

Based on the toxicological identification of drugs per case, MRDs were classified into four categories, as follows: pure Methadone findings (M), findings with Methadone and Benzodiazepine (M+B), findings with Methadone and Other drugs excluding benzodiazepines (M+O), and findings with Methadone, Benzodiazepines and Other drugs (M+B+O). The distribution of the cases among the four categories was: 11.1% for M, 40.7% for M+B, 3.7% for M+O and 44.4% for M+B+O. The median (range) blood concentration of methadone in M category was 0.11 µg/ml (0.02-0.14µg/ml). Furthermore, the median (range) blood concentrations of methadone and diazepam in M+B category were 0.11µg/ml (0.02-0.39µg/ml) and 0.07µg/ml (0.02-0.41µg/ml), respectively. A total of 22 non-benzodiazepine drugs in M+B+O group were detected. Sertraline (antidepressant), clozapine (antipsychotic), non-steroidal anti-inflammatory drugs (acetaminophen, ibuprofen) and tramadol (weak opiate analgesic) were the most frequently registered. Alcohol was present in blood in 13 cases with median value of 0.71mg/ml (range 0.13-2.02mg/ml).

The observed increased frequency of MRDs in the region of Vojvodina showed that most of the deceased used methadone in combination with benzodiazepines (sometimes with other psychoactive medications) on their own initiative. Both the median and the highest detected concentrations of methadone and diazepam were within the therapeutic range.

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Methadone-Related Deaths, Methadone, Benzodiazepines

K15 A Remarkable Case of Fatal Monocrotophos Poisoning by Cutaneous Absorption While Sleeping

Jatin Bodwal, MBBS, MD, Deen Dayal Upadhyay Hospital, Dept of Forensic Medicine, Hari Nagar, New Delhi 110064, INDIA; and Seema S. Sarohe, MeD, Central Institute of Education, 33, Chattra Marg, University of Delhi, New Delhi 110007, INDIA*

After attending this presentation, attendees will better understand how monocrotophos can elicit toxicity by causing chemical burns and systemic manifestations, which in this case occurred while sleeping.

This presentation will impact the forensic science community by describing a rare case of fatal monocrotophos poisoning due to its indiscriminate use in India, which is taking innocent lives; this case also reinforces a long-standing demand for its complete ban.

Monocrotophos is commonly used by debt-ridden farmers for committing suicide in India. Recently, many children in a school died due to monocrotophos poisoning after they consumed a midday meal which had been cooked in an empty container of monocrotophos. No case has previously been reported of a fatal, accidental monocrotophos poisoning through skin exposure while sleeping in a household.

In this case, a middle-aged woman sprayed diluted monocrotophos in her house to avoid a mosquitos, then slept on the floor while keeping the bottle of monocrotophos at hand. During the night, the bottle of monocrotophos accidentally fell over and the contents of the bottle slowly reached the sleeping woman, which caused cutaneous absorption of monocrotophos. Early in the morning, acute organ phosphorus poisoning symptoms forced her to be admitted to the hospital. She died after a day of treatment and her body was transferred for autopsy. Oddly, no other family member was affected. This puzzle was solved during the autopsy.

At autopsy, chemical burn patches were found on the back of the right arm and shoulder. These burns were not noticed during treatment and were only revealed after her clothes were removed during the autopsy. Viscera, blood samples, and affected skin samples were preserved.

In the laboratory, a blood sample was deproteinized by using 5ml of sodium tungastate solution and a few drops of sulphuric acid, then shaken for few minutes and filtered. The filtrate was transferred into a separating funnel and extracted with acetone. Skin samples were pretreated with 15ml-20ml of acetone. The mixture was shaken at intervals, kept overnight, and filtered. Stomach and intestine tissues were homogenized with acetone using a high-speed blender, kept overnight, then the solvent was decanted from the tissues the next day. The decanted solvent was treated with anhydrous sodium sulphate to remove water content. Tissues from the liver, spleen, and kidney were mixed with ammonium sulphate and acetic acid. The mixture was shaken at intervals, kept overnight for digestion, and filtered. The extract was transferred to a separating funnel and extracted with acetone. The layer was then treated with anhydrous sodium sulphate.

Pink-colored spots were developed in a Thin-Layer Chromatography (TLC) plate on the area where the monocrotophos was separated. After TLC, Gas Chromatography/Mass Spectrometry (GC/MS) analysis was performed using a benchtop GC/MS system which consisted of an Agilent® 6890N gas chromatograph interfaced to an Agilent® 5975 inert XL mass selective detector with an auto sampler. In GC/MS, retention time and mass spectra of the exhibits were compared with the standard, and blood and skin samples were matched.

In India, deaths due to organophosphates are very high. A study by Rao et al. revealed that 91 people died due to monocrotophos poisoning during a five-year (1997-2002) period out of a total of 8,040 poisoning cases reported in the southern part of India.[1] In another study sponsored by the World Health Organization (WHO), 1,531 poisoning cases were reported in different states of India during the year 1999-2000, out of which 86 cases were attributed to monocrotophos. In countries such as Sri Lanka, 72% of the total cases of poisoning were caused by organophosphates in the year 1991-1992; consequently, monocrotophos was banned in 1998. In countries such as Indonesia and Brazil, a total of 214 cases and 107 cases were reported, respectively, in different studies.

The Food and Agriculture Organization (FAO) and WHO have encouraged countries to phase out highly hazardous pesticides. Leading Asian countries have banned the use of monocrotophos because of unacceptable health risks; however, in India, monocrotophos continues to be produced, used, and exported.

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Monocrotophos, Poisoning, India

K16 Detection of Metal Phosphide Poisoning by Using Headspace/Gas Chromatography With Flame Ionization Detector (HS/GC/FID)

Sardar Ali Wattoo, MPhil, Punjab Forensic Science Agency, Old Multan Road Thokar Niaz Baig, Lahore, Punjab 53700, PAKISTAN; Muhammad Taimoor Chaudhary, MPhil, Punjab Forensic Science Agency, Thokar Niaz Baig Multan Road, Lahore, Punjab, PAKISTAN; and Mohammad A. Tahir, PhD, Punjab Forensic Science Agency, Thokar Niaz Beig, Multan Road, Lahore, PAKISTAN*

After attending this presentation, attendees will better understand the potential of Gas Chromatography (GC) in qualitative and quantitative analysis of biological samples for highly noxious and fatal phosphine gas. This presentation will also help to highlight the increasing incidences of phosphine poisoning, thereby bringing attention to government agencies for possible legislation regarding its use.

This presentation will impact the forensic science community by introducing an easy, economical, and reliable means of phosphine analysis from biological specimens. The technique can be used for postmortem toxicology analysis of gastric contents as well as in antemortem cases of gastric lavage.

Metal phosphides, particularly aluminium phosphide, zinc phosphide, and magnesium phosphide are used as rodenticides and fumigants. It has been found that aluminium phosphide poisoning cases are increasing in Pakistan and some other Asian countries, particularly in suicide attempts. Metal phosphides, after conversion to phosphine gas in the body, produce severe organ damage. The mortality rate with metal phosphide poisoning is very high as no antidote is available.

A method for the detection of metal phosphide poisoning was developed using HS/GC/FID. Phosphine gas was detected and quantitated from the gastric contents of persons who died of aluminium phosphide ingestion (either suicidal or homicidal). The samples were mixed with sulfuric acid in HS vials, the vials were crimped immediately, then analyzed by using the HS/GC technique with four point calibration (503µg/g-1,000µg/g). The method was validated by analysis of spiked simulated gastric contents and was found to be linear in the working range of 10µg/g to 2,000µg/g. Zinc phosphide was used as standard reference material and the amount of phosphine produced from zinc phosphide was calculated stoichiometrically. Limit Of Detection (LOD) of phosphine was 3µg/g, Limit Of Quantitation (LOQ) was 10µg/g, and accuracy and precision at three different concentrations (50µg/g, 100µg/g, and 1,000µg/g) were within the acceptable range (accuracy: 92.6%-111.6% and %CV: 14.6%-18%).

Phosphine, Metal Phosphides, Headspace Chromatography

K17 Vitreous Humor Chemistry of Heroin-Related Deaths as Compared With the General Population of Non-Drug-Related Deaths in the City and County of San Francisco From 2010 Through 2013

Glenda M. Easterling, BS, OCME, 850 Bryant Street, San Francisco, CA 94103; Pavlos Karamanidis, BS, 407 Sanchez Street, Apt 3120, San Francisco, CA 94114; Eric A. Ingle, BA, 1965 Pleasant Hill Road, Pleasant Hill, CA 94523; Chinyere M. Williams, BS, 2527 8th Avenue, Apt 211, Oakland, CA 94606; Jeffery Hackett, PhD, OCME, Forensic Laboratory Division, 850 Bryant Street, N Terrace, San Francisco, CA 94103; and Nikolas P. Lemos, PhD, OCME, Forensic Lab Division, Hall of Justice, N Terrace, 850 Bryant Street, San Francisco, CA 94103*

After attending this presentation, attendees will better understand the vitreous humor chemistry results of heroin-related deaths as compared to the vitreous humor chemistry results of the general population of decedents who had no drugs detected at the San Francisco Office of the Chief Medical Examiner from 2010 to 2013.

This presentation will impact the forensic science community by offering the forensic community a deeper insight into the relative vitreous humor chemistry in heroin-related deaths and non-drug-related death populations in the city and county of San Francisco.

Heroin (diacetylmorphine) is metabolized to 6-monoacetylmorphine, which is frequently a target analyte in forensic toxicology laboratories as confirmation of heroin abuse. One of the most popular specimens employed for this confirmation in recent times is vitreous humor. This fluid is often examined for the concentrations of electrolytes and compounds such as sodium, potassium, chloride, glucose, vitreous urea nitrogen, and creatinine. The Forensic Laboratory Division at the Office of the Chief Medical Examiner in San Francisco routinely tests vitreous humor on behalf of the Pathology Division for signs of diabetic ketoacidosis, dehydration, and general electrolyte imbalance. Review of the data involving both sets of information (i.e., presence of opioids and vitreous chemistry) may assist analysts in relating to these types of deaths in comparison to non-opioid-related fatalities.

Method: A meta-analysis was performed on the vitreous humor chemistry data obtained from previously analyzed specimens in both heroin-related deaths and those from non-drug-related deaths to determine if there exists a direct relation between heroin death and the results obtained during postmortem chemistry studies using a sample population from the City and County of San Francisco. This was performed by accessing data obtained from a commercially available spread sheet data base populated by results obtained by staff at the Forensic Laboratory Division. The hypothesis is that there exists a significant difference in vitreous humor chemistries between the two populations; whereas a null hypothesis suggests there would be no significant differences in terms of electrolyte concentrations in the populations.

Data was reviewed from the analyses performed using a vitreous humor chemistry analyzer employed at the Forensic Laboratory Division and the San Francisco Office of the Chief Medical Examiner. This database was interrogated from the period of 2010 to 2013. This data included the determined concentrations of sodium, potassium, chloride, glucose, vitreous urea nitrogen, and creatinine, the presence of 6-monoacetylmorphine, age, gender, as well as cause and manner of death. A total of 5,190 cases were reviewed. Exclusions were applied to those deaths of subjects less than 16 years of age and greater than 65 years of age, and in those cases in which the potassium concentrations were greater than 30mmol/L.

Cases for inclusion listed 6-monoacetylmorphine or diacetylmorphine in the Forensic Laboratory Division's and the Medical Division's databases. From this review of the cases and the respective electrolyte concentrations in vitreous humor, the data was exported to commercially available spreadsheet software and Analysis of Variance (ANOVA) was used for statistical calculations and assessment.

Results: The Pathology Division of the Office of the Chief Medical Examiner requested vitreous humor chemistry analysis in 8 of 36 heroin-related deaths. The control samples were comprised of 128 randomly selected, non-drug-related deaths. The concentrations of sodium ranged from 72mmol/L to 146mmol/L in the heroin-related deaths, while the range was 92mmol/L to 207mmol/L for non-heroin-related deaths. In the case of chloride concentrations, 65mmol/L to 125mmol/L were found in heroin-related deaths; the corresponding values in the non-heroin-related cases were found to be 70mmol/ L to 138mmol/L. From this data, it was determined that both sodium and chloride showed statistical significance ($p<0.05$) using 95% confidence interval when comparing heroin-related deaths to the control population. Most other electrolytes did not show any statistically significant differences. Glucose and ketone were not analyzed for variance because in almost all of the heroin-related deaths, glucose results were <25mg/dL and ketones were almost always negative.

Conclusion: In this three-year study comparing the vitreous humor chemistry of heroin-related deaths to a control population of non-drug-related deaths in the City and County of San Francisco, it was found that the relationship between sodium and chloride concentrations showed statistically significant differences between the two populations. If poor nutrition or dehydration in the opioid population was a controlling factor, one would expect to see elevation in sodium or chloride concentrations, which is not being observed. The statistical evaluation of the concentrations of other vitreous humor electrolytes will remain the subject of further studies at the Forensic Laboratory Division of the San Francisco Office of the Chief Medical Examiner.

Vitreous Humor Chemistry, Heroin, Deaths

K18 A European Rave Drug (Prolintane) Fatality in Phoenix, Arizona

Whitney Brown, BS, Maricopa County Office of the Medical Examiner, 701 W Jefferson Street, Phoenix, AZ 85007; Ian Duffy, BS, RK Clinical Solutions, LLC, 145 S 79th Street, Ste 30, Chandler, AZ 85226; and Norman A. Wade, MS, OME, Forensic Science Center, 701 W Jefferson Street, Phoenix, AZ 85007-2908*

After attending this presentation, attendees will have a basic knowledge of prolintane and its danger as a synthetic sympathomimetic amine that is being used in the European Rave scene and is emerging in the United States.

This presentation will impact the forensic science community by discussing a specific case example of a drug that has been around since the 1960s in Europe, yet is just starting to show up in isolated cases in the United States.

Prolintane (phenylpyrrolidinopentane) is a synthetic sympathomimetic amine that was originally introduced in the 1960s as a treatment for narcolepsy and attention deficit disorder.[1] Although prolintane continues to be prescribed in Europe, Africa, and Australia, it is not legally available in the United States.[1] It has been compounded with multivitamins and made available in tonic or tablet form where prescribed, though it has been used as a doping agent worldwide; for this, it has been banned by the National Collegiate Athletic Association and the World Anti-Doping Agency.[1] It is not scheduled by the United States Drug Enforcement Agency.

Blood or plasma levels of prolintane following oral administration have not been reported. The adverse effects of this drug may include headache, anxiety, hypertension, tachycardia, anorexia, and insomnia.[2] The literature reports experiences of dizziness, palpitations, hyperactivity, and a panic reaction along with confusion, agitation, loss of consciousness, and combativeness.[1] Prolintane undergoes extensive biotransformation with the production of at least 18 metabolites.[2]

In 2007, Kyle and Daley reported two cases in Mississippi of the first medically documented cases of prolintane abuse in the United States (both victims survived the over-dosage).[1] Testing on both cases was completed on urine only with Solid Phase Extraction (SPE), then with Gas Chromatography/Mass Spectrometry (GC/MS) analysis.

In the presented case, a 51-year-old man was observed running through traffic and acting strangely. The subject was tackled by police and eventually handcuffed, but then went unresponsive while in police custody. The officers did not use a Taser™ nor any injuring force. The subject was rushed to the nearest emergency room but sustained rapidly progressive respiratory distress, then acute cardiopulmonary arrest. Resuscitative efforts were not successful. A urine drug screen in the hospital was found to be positive for PCP and Ecstasy. Upon preliminary standard protocol of postmortem examination, no immediate cause of death could be attributed except for hyperthermia of 102.7°, diffuse pallor, and dry mucous membranes.

The decedent's hospital samples were tested for alcohols by Gas Chromatography/Flame Ionization Detector (GC/FID) and for common drugs of abuse by **Enzyme-Linked Immuno-Sorbent Assay (**ELISA), with an unknown peak detected in the basic drug screen of the blood by Gas Chromatography/Nitrogen Phosphorous Detection (GC/NPD) and confirmation by GC/MS. A sample of verified standard prolintane was obtained from the University of Mississippi and the identity of the unknown peak was verified. Prolintane was quantitated by GC/MS and found to be 1.50mg/L in the hospital blood. No other drugs or volatile compounds were detected in the hospital samples, including PCP and MDMA, by instrumental analysis including Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).

The deputy chief medical examiner performed a comprehensive autopsy on the decedent and came to the conclusion that the cause of death was due to complications of hyperthermia associated with acute prolintane intoxication and physical exertion in an outdoor heat environment.

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Prolintane, Postmortem, Toxicology

K19 Dangers of Carbon Monoxide (CO) Generated From Small Internal Combustion Engines

Sandra Bishop-Freeman, PhD, NC OCME, 4312 District Drive, Raleigh, NC 27607; Marc Feaster, BS*, NC-OCME, 4312 District Drive, Raleigh, NC 27607; Franklin Pippen, BS, 112 Meadowview Place, Lenoir, NC 28645; and Ruth E. Winecker, PhD, OCME, 3025 Mail Service Center, Raleigh, NC 27699-3025*

After attending this presentation, attendees will have greater insight into the different types of small engines capable of generating CO, the technology behind detection devices used by first responders, and the symptoms of CO toxicity.

This presentation will impact the forensic science community by providing information regarding case studies in which CO generation came from unexpected sources. Interestingly, the specificity of sensors used in hand-held detectors may not be selective enough to rule out interfering gases when the source of CO is under investigation.

Small engines and tools have the danger of emitting potentially lethal concentrations of CO if used in a poorly ventilated environment. Forklifts, pressure washers, gas pool heaters, propane-powered construction tools, and lawn mowers are some examples. In fact, efforts are underway, through industrial commissions, to retro-fit forklifts with catalytic convertors or three-way catalytic mufflers for Liquid Petroleum Gas (LPG) engines to reduce emissions, thus increasing worker safety.

The North Carolina Office of the Chief Medical Examiner encountered a case in which a 57-year-old male was found deceased in his air-conditioned garage. The death originally presented as a probable sudden natural death due to a history of hypertension. When analysis revealed a lethal concentration of 55% Carboxyhemoglobin (COHb) saturation in the decedent's blood, the toxicologist immediately informed the medical examiner and law enforcement for further investigation. First responders were initially misled when the Dräger X-am® 2000 gas detector gave a positive reading for CO when operated next to an acetylene/oxygen welding torch and tank assembly. The local fire department was unaware of the cross sensitivities of the device, which will cause erroneous detection if exposed to certain concentrations of other small molecular gases. The actual source of CO was later determined to be a gasoline-powered pressure washer kept inside the garage. A few months later, a public health emergency was investigated in a North Carolina grocery store when a worker passed out and several employees felt ill. The cause was later determined to be toxic levels of CO generated by a propane-powered tile cutter that ultimately sent 17 people to the hospital for treatment.

These and additional cases will be explained in detail in order to educate the forensic community about the dangers of CO produced by engines more esoteric than the automobile. Along with a review of gas-sensing technologies and the signs and symptoms of toxicity, this presentation will be a comprehensive assessment of the hazards of small equipment-generated CO emissions.

Carbon Monoxide, Death Investigation, Toxicology

K20 A Retrospective Analysis of Deaths Due to Carbon Monoxide (CO) Poisoning Reported at a Tertiary Care Center in New Delhi, India, From January 2010 to January 2015

Shivani Dhaka, MBBS, AIIMS, New Delhi, Dept of Forensic Medicine, AIIMS, New Delhi, Delhi, INDIA; Sudhir K. Gupta, MD, AIIMS, Dept of Forensic Medicine & Toxicology, New Delhi, New Delhi 110029, INDIA; Chittaranjan Behera, MD, Department of Forensic Medicine, AIIMS, Ansarinagar, New Delhi 110029, INDIA; and Rajanikanta Swain, MD, All India Institute of Medical Sciences, Rm No-93, Hostel N0-8, AIIMS, Ansari Nagar, New Delhi, Delhi 110029, INDIA*

After attending this presentation, attendees will better understand suspected cases of CO poisoning depending on history, environment in which the deceased is found, and various findings at the time of autopsy.

This presentation will impact the forensic science community by helping attendees understand the various autopsy/pathological findings that are seen in a typical case of CO poisoning as well as obscure findings that are often missed or confused with other causes of death as these are quite atypical. This study will have a greater implication over large metropolitan cities of India, where large numbers of migrating individuals are living in overcrowded shelters. Though being significantly at risk, they still remain oblivious to the fatal nature of such a poisoning. The sudden and incumbent nature of this fatality is not well reported or dealt with in the subcontinent, thus resulting in a preventable loss of life. The suddenness of the events leave the relatives of the deceased unable to cope with this sudden and seemingly inexplicable demise.

CO poisoning is one of the leading causes of poisoning deaths in the United States, accounting for approximately 40,000 emergency department visits and 5,000-6,000 deaths per year, a significant number of which are accidental and thus preventable.[1,2] These types of deaths are relatively uncommon in tropical countries, like the subcontinent of India, where artificial heating and closed habituation are not quite as prevalent. But, with changing social demographics and the adoption of modern household technology, the need for awareness has also increased. The sources of CO may be as innocuous as smoke and fumes from gas geysers, stoves, water heaters, burning oil lamps, etc.; products that are used in common households which do not have an actual visible flame. Another common source, typically used for suicidal CO poisoning, are automobile exhaust fumes.

CO is a colorless, odorless, lighter-than-air gas, whose level in ambient air, when less than 0.2ppm, is not harmful to humans. There are usually no signs or symptoms between the levels of 1ppm to 70ppm.[3] CO poisoning is a type of hypoxic condition. CO binds to hemoglobin with 250 times more affinity than oxygen.4 Thus, even if oxygen is available in the environment, the body is unable to utilize it (best explained by the age-old adage: "Water, water, everywhere, Nor any drop to drink"), a condition classified as anemic hypoxia.[5] The acutely affected subject becomes lethargic and is unable to take appropriate action.

A total of 28 cases of death due to CO poisoning (accidental and suicidal) brought to the All India Institute of Medical Sciences (AIIMS) mortuary over a period of five years, between January 2010 and January 2015, were analyzed. Of these, 82.1% of the cases were from a low socio-economic status. This may be due to the fact that in sub-urban regions of India, coal, wood, and cow-dung cakes are used as a source of heat; this conclusion was strengthened by crime scene visits. Cherry red discoloration was present in 42.9% of the cases, and blisters containing pink fluid were present on the calves and buttocks in 28.6% of the cases. Surprisingly, serous effusions such as pleural and pericardial effusions were seen in 64.3% of the cases and generalized congestion was observed in nearly all cases. Apart from CO, alcohol was present in 39.3% of the cases, with an average of 162.3mg%. Toxicological analysis of the viscera report was negative in 7.1% of the cases, but circumstantial findings, such as partially burnt coal or wood, were present in a poorly ventilated rooms.

To conclude, this study will enable attendees to become more aware and have better insight into the circumstances and autopsy findings in suspected cases of death due to CO poisoning. This study also sheds light on the need for specific protocols that should be followed in all suspected cases of CO poisoning. Also, this study will inspire the forensic community to plan and implement various steps required to educate the masses regarding the prevention of CO poisoning.

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Carbon Monoxide, Poisoning, Postmortem

K21 The Analysis of Benzodiazepines in Dried Blood Spots (DBS) Using Liquid Chromatographic/ Tandem Mass Spectrometry (LC/MS/MS)

Andrea L. Jones, BS, Cedar Crest College, 2819 Fernor Street, Apt 307, Allentown, PA 18103; and Thomas A. Brettell, PhD, Cedar Crest College, 100 College Drive, Allentown, PA 18104*

After attending this presentation, attendees will better understand the use of DBS as a substrate for collection of blood for the purpose of detecting specific benzodiazepines.

This presentation will impact the forensic science community by providing a method that determines the sensitivity and stability of common benzodiazepines in blood samples using FTA®DMPK-C (untreated) cards by GE Healthcare as the substrate.

Benzodiazepines are central nervous system depressants and are commonly prescribed medications in the United States today. They are classified as Schedule IV in the Controlled Substances Act and have a high potential for abuse due to sedative properties, especially when mixed with other depressants. This is important because the detection of benzodiazepines is pertinent to Driving Under the Influence (DUI) toxicology. Drug-impaired drivers harm or kill thousands of people each year in the United States and there is a growing body of scientific evidence confirming that driving under the influence of prescribed medications has become a significant problem worldwide. Therefore, a selective and sensitive analytical method for the detection and stability of benzodiazepines in biological samples would be exceedingly beneficial to the field of forensic toxicology. Among the benzodiazepines available, analyses of alprazolam, clonazepam, 7-aminoclonazepam, diazepam, lorazepam, nordiazepam, nitrazepam, and flunitrazepam in blood samples in addition to the deuterated internal standards alprazolam-d5, clonazepam-d4, 7-aminoclonazepam-d4, and lorazepam-d4 have been used in this research utilizing DBS.

The use of DBS on cards as a substrate for collection of blood for the purpose of detecting drugs in DUI cases has many advantages. The technique uses less blood, typically 10µL-50µL of capillary blood, which can be obtained through minimally invasive procedures. The cards are easy to handle, easy to transport, and can be stored at ambient temperature in the laboratory with minimal analyte loss. This makes sampling simpler, faster, and less invasive.

A selective LC/MS/MS method was developed for the analysis of eight benzodiazepines. The method showed good linearity for each benzodiazepine with R^2 values of >0.99 and was able to detect down to 50ng/mL of each analyte in 10µL of blood from a DBS with a simple methanol extraction. The analysis was performed using an ABI® SCIEX™ 3200 Qtrap® triple quadrupole mass spectrometer interfaced with a Shimadzu® LC system consisting of two Shimadzu® LC-20AD Prominence liquid chromatography binary pumps, two Shimadzu® DGO-20A_3 Prominence degassers, and a Shimadzu® SIL-20AC Prominence auto sampler. Chromatographic separation was achieved using an Ultra® Biphenyl LC Column (5.0cm x 3.0mm i.d., 2.7µm particle size). The High-Performance Liquid Chromatography (HPLC) method was isocratic with 30:70 0.1% (v/v) formic acid in methanol and the total run time was 9.50 minutes. A retention time versus temperature optimization study provided the most favorable separation conditions at 20°C.

Benzodiazepines, Dried Blood Spots, LC/MS/MS

K22 Detecting Ketamine in the Hair of Buried Decomposed Rats Using Liquid Chromatography/ Tandem Mass Spectrometry (LC/MS/MS)

Christine Barrett, BS, Arcadia University, 450 S Easton Road, Glenside, PA 19038; Kimberlee S. Moran, MSc, Forensic Outreach, 231 Cedarbrook Road, Sicklerville, NJ 08081; Gail Cooper, PhD, Forensic Medicine and Science, University Place, Glasgow, Scotland G12 8QQ, UNITED KINGDOM; and Karen S. Scott, PhD, Arcadia University, 450 S Easton Road, Glenside, PA 19038*

After attending this presentation, attendees will understand the effects of burial and decomposition on the detection of drugs of abuse and their metabolites using hair as the biological matrix.

This presentation will impact the forensic science community by providing education on the utility of the hair matrix and showing the relationship between decomposition and drug detection that could be of use in future research and casework.

Although many studies have been conducted to detect and identify drugs in typical biological matrices of decomposing remains, few have concentrated on hair as a matrix. Being able to use hair as a matrix is very beneficial due to the stability of retaining drugs and the fact that it does not easily degrade in different environmental conditions. Understanding how to utilize hair in postmortem toxicology is important because it often outlasts other body matrices and provides a long-term picture of an individual's drug use.

The main objective of this project was to determine if postmortem interval in a buried environment affected the concentrations of the parent drug and metabolites (norketamine and dehydronorketamine) in hair and if any trends related to dosage or decompositional stage were present. Additionally, concentrations of ketamine and metabolites were compared in white hair versus black hair for all possible rats.

Rats were dosed at concentrations of 20mg/kg (n=13), 30mg/kg (n=13), and 40mg/kg (n=13) for ten consecutive days. The rats were then either euthanized an hour after the last injection (n=21) or ten days after the last injection (n=15). The rats were then buried in the New Jersey Pine Barrens where they stayed for a total of either 77, 188, 293, 793, or 3,104 Accumulated Degree Days (ADDs), spanning from January to October 2014. Four non-drugged rats were used as controls along with six rats that were dosed (10mg/kg, 15mg/kg, 20mg/kg) but not buried.

Hair was removed from the pelts and divided into dark hair, light hair, shaved hair, and plucked hair. In order to remove any decomposition fluid and soil, hair samples were washed under sonication with Deionized (DI) water for a minimum of three times at five minutes each, and then with dichloromethane three times at five minutes each. The hair was then dried and cut up to optimize the extraction of the drug, weighed to 15mg in triplicate, then extracted with an acidic methanolic extraction (50:1 MeOH:HCl). Controls were produced by spiking washed, drug-free hair with a working solution of ketamine, norketamine, and dehydronorketamine.

As in previous studies, black (pigmented) hair had higher concentrations of drug compared to the white (non-pigmented) hair of the corresponding sample for the control (unburied) rat; however, data from this research has shown that burial affects this trend. Ketamine and metabolite concentrations increased with an increase of decomposition, most likely due to postmortem fluid contamination. For rats that were killed immediately after the ten-day dosage, norketamine had higher concentrations than ketamine, with dehydronorketamine having a low response. For rats that were killed ten days after the final injection, ketamine had a similar concentration to norketamine, with dehydronorketamine again having a low response.

Ketamine, Hair, Postmortem Toxicology

K23 Detection, Quantification, and Relative Distribution of Ketamine, Norketamine, and Dehydronorketamine in Skeletal Tissue of Dosed and Buried Rat Remains at Different Stages of Decomposition

Kimberlee S. Moran, MSc, Forensic Outreach, 231 Cedarbrook Road, Sicklerville, NJ 08081; James Watterson, PhD, Laurentian University, 935 Ramsey Lake Road, Sudbury, ON P3E 2C6, CANADA; Karen S. Scott, PhD, Arcadia University, 450 S Easton Road, Glenside, PA 19038; and Erica N. Johnson, BA, Arcadia University, 450 S Easton Road, Glenside, PA 19038*

After attending this presentation, attendees will be informed of the potential to detect ketamine in buried skeletal tissue from decomposing rat remains using a passive methanolic extraction, solid phase extraction, and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method. In addition, attendees will understand the effect of postmortem interval on drug detection, quantification, and relative drug distribution.

This presentation will impact the forensic science community by demonstrating the effects of burial and postmortem interval on levels of ketamine, its metabolites, and their relative distribution in skeletal tissue, providing toxicologists with a reason to analyze buried and decomposing remains.

During a death investigation in which decomposition has occurred, skeletal remains may be the only available source of toxicological information. Recent literature has explored drug detection in skeletal tissue, but implications of drug measurements in bone as well as drug distribution remain poorly understood. Additionally, few studies have been published on buried remains and remains at different stages of decomposition.

The goal of this study was to detect and quantify ketamine in buried skeletal tissue from different stages of decomposition and to determine what bones are best suited for toxological analysis. Rats were dosed daily for ten days at three levels: 20mg/kg (*n*=13), 30mg/kg (*n* 13), and 40mg/kg (*n* 13). Control rats (*n* 3) remained untreated. Rats were euthanized in two groups: one hour after the last injection (*n* 21) and ten days after the last injection (*n* 15). One rat from each dose group (0mg/kg, 20mg/kg, 30mg/kg, and 40mg/kg) was left unburied and tested for drugs. The remaining rats were buried in the New Jersey Pine Barrens and exhumed at different stages of decomposition (Fresh, Early Decomposition, Advanced Decomposition, and Skeletonization). Accumulated Degree days (ADD) were used to calculate the decomposition stage (77, 188, 293,793, and 3104 ADDs). Rats were dissected and organs, skin, and hair were removed. Bones were cleaned of remaining tissue using dermestid beetles. By 3,104 ADDs, rats were fully skeletonized and not introduced to the beetles. Bone types were separated (pelvis, vertebrae, upper limbs, and lower limbs) and sampled (500mg) to be ground using a Biotage® Bead Ruptor 24. Samples underwent passive methanolic extraction and solid phase extraction. Ketamine, norketamine, and dehydronorketamine were detected in skeletal tissues using LC/MS/MS with ketamine-D4 as the internal standard. Drug levels were compared across decomposition stages and bone types.

Consistent with the hypothesis, drugs were not detected in the control samples. Ketamine, norketamine, and dehydroketamine were detected in skeletal tissues of the unburied subjects and most buried subjects that were dosed. Detected levels were higher in rats that received higher doses. Ketamine and its metabolites were detected in most bone types, with higher levels in long bones as a result of there being more marrow and blood flow. Due to the ability to detect ketamine in skeletal tissues, toxicologists should attempt drug screening, even at advanced stages of decomposition.

Ketamine, Bone, LC/MS/MS

K24 Comparison of Cocaine Concentrations in Heart Blood, Thigh Muscles, and Thigh Bones

Ken-ichiro Nakao, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, JAPAN; and Kazuhiko Kibayashi, MD*, Tokyo Women's Medical University, Dept of Legal Medicine, School of Medicine, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, JAPAN*

After attending this presentation, attendees will understand the interpretation of cocaine concentrations in bone tissue of decomposed or skeletal remains.

This presentation will impact the forensic science community by demonstrating the role and importance of toxicological analysis of bone in the diagnosis of death from drug abuse.

Bone samples are used for toxicological analysis of decomposed or skeletal remains. To determine the relationship between the concentrations of cocaine in bone and those in blood or muscle, the cocaine concentrations in mouse thighbones were studied and these concentrations were compared with those in heart blood and thigh muscles.

Male ddY mice were intraperitoneally injected with cocaine at doses of 5mg/kg, 15mg/kg, or 30mg/kg or with saline, once a day for seven days (*n*=5 per group). Heart blood samples were collected under general anesthesia by cardiac puncture, and the thigh muscles and thighbones were also removed. Five hundred microliters of heart blood, 0.5g of thigh muscle, or 0.2g of thighbone were analyzed using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). Thighbone samples were sterilized with distilled water and acetone and were dried at 50°C for 24 h. The dried thighbones were pulverized with a bead homogenizer and were evaluated using LC/MS/MS.

In all mice groups, cocaine concentrations in thighbone samples were higher than those in heart blood and thigh muscle samples. Significantly higher concentrations of cocaine were determined in thighbone samples of mice administered a dose of 15mg/kg or 30mg/kg cocaine ($p < 0.05$). The range of correlation coefficients of cocaine concentrations between thighbones and heart blood, or thighbones and thigh muscle, were 0.870-0.986 in all mice groups. The results of this study indicated that: (1) cocaine shows higher concentrations in bone than in blood or muscle; and, (2) the cocaine concentrations in thighbone and heart blood or thigh muscle had strong correlation. Therefore, this study indicated that cocaine used at the time of death can be detected by cocaine analysis from a bone, and it may be able to estimate the concentrations of cocaine in the heart blood and thigh muscle from the cocaine concentrations in thighbones under continued cocaine use.

Cocaine, Bone, Mouse

K25 Methamphetamine, Amphetamine, and Norephedrine Levels in Dermestid Beetles From the Consumption of Dosed, Buried Rat Remains

Meaghan P. Drumm, 450 S Easton Road, Glenside, PA 19038; M. Lee Goff, PhD, Chaminade Univ of Honolulu, Forensic Sciences Program, 3140 Waialae Avenue, Honolulu, HI 96816-1578; Karen S. Scott, PhD, Arcadia University, 450 S Easton Road, Glenside, PA 19038; and Kimberlee S. Moran, MSc, Forensic Outreach, 231 Cedarbrook Road, Sicklerville, NJ 08081*

After attending this presentation, attendees will understand the potential to detect amphetamine and methamphetamine and their metabolites in dermestid beetles using a homogenization, solid phase extraction, and a Gas Chromatography/Mass Spectrometry (GC/MS) method. Attendees will also learn the effects of postmortem interval on drug detection and the best developmental stages (larvae, pupae, adult, and frass) for drug detection in dermestid beetles.

This presentation will impact the forensic science community by providing information on the use of dermestid beetles for the detection of drugs even at advanced stages of decomposition. This research identifies the best dermestid beetle developmental stage for detecting amphetamine and its metabolites when no other tissue remains.

There are multiple studies detecting various drugs within blow flies. Few studies have attempted to detect and quantify drugs within other insects such as dermestid beetles (Order: Coleoptera). These beetles are the last insects found feeding on decomposing remains. To date, the only reported studies dealing with Coleoptera have been on beetle exuviae, from which amitriptyline and nortriptyline were isolated.

It was determined that methamphetamine and amphetamine could be detected in four beetle developmental stages fed on buried rats dosed with 10mg/kg (*n*=4), 6mg/kg (*n*=4), 2mg/kg (*n*=4), 1mg/kg *n* 2), 0.6mg/kg (*n*=1), and 0.2mg/kg (*n*=2), amphetamine, and 5mg/kg (*n*=4), 3mg/kg (*n*=4), 2.5mg/kg (*n*=2), 1.5mg/kg (*n*=2), 0.5mg/kg (*n*=4), and 0.25mg/kg (*n*=2) methamphetamine. Three control rats were injected with saline and buried and two control rats were not injected and not buried. All rats were euthanized with Carbon Dioxide (CO_2) gas prior to burial. Buried rats were exhumed at different decomposition stages (89, 182, 395, and 819 Accumulated Degree Days (ADDs)) to determine the effects of decomposition on the ability to detect the drugs using GC/MS. After exhumation, rats were dissected, pelted, and dried before being fed to dermestid beetles (*Dermestidae maculatus*). Eight adult beetles were placed on each sample until they laid eggs, then removed. All offspring consumed meat of the dosed rats until only bones remained. Remaining adult beetles, larvae, and pupae were collected and homogenized using a mortar and pestle and liquid nitrogen; beetle frass was also collected. Samples were centrifuged and drugs extracted using solid phase extraction. The amounts of methamphetamine, amphetamine, and norephedrine were quantified in all beetle media using GC/MS with methamphetamine D5 and amphetamine D5 as internal standards.

As predicted, methamphetamine, amphetamine, and norephedrine were detected in all beetle mediums (larvae, pupae, adult, and frass). All non-drugged controls were negative. Samples that contained drugs exhibited a dose response relationship curve. This means that the higher the concentration of drug in the rat, the more that methamphetamine, amphetamine, and norephedrine were able to be detected in beetle mediums. Of all the mediums, frass was the easiest to handle while pupae were the hardest to collect since they were the most fragile.

Methamphetamine, Dermestid Beetles, GC/MS

K26 Determination of Drug Distribution in Postmortem Tissues and Bones of Pigs Administered Drugs

Ismail E. Goren, BS, Cukurova University, Dept of Forensic Medicine, Balcali, Adana 01330, TURKEY; Nebile Gokce Daglioglu, PhD, Cukurova University, Faculty of Medicine, Dept of Forensic Medicine, Adana 01130, TURKEY; Mete K. Gulmen, PhD, MD*, Cukurova University, School of Medicine, Dept of Forensic Medicine, Adana 01330, TURKEY; and Pinar Efeoglu, MS, Cukurova University School of Medicine, Dept of Forensic Medicine, Balcali, 01330, Adana 01330, TURKEY*

After attending this presentation, attendees will better understand whether organ and bone samples collected from decomposed and buried corpses at different postmortem intervals are useful for predicting the blood concentration of drugs.

This presentation will impact the forensic science community by clarifying that analyses on body tissues and interpretation of results after decomposition and exhumation are challenging tasks. This presentation will add to research being conducted regarding how the concentration of drugs change during burial time and exhumation and how this concentration may depend on the type of bone.

Forensic toxicological analyses may provide significant information in determining the cause of death or in *explaining forensic cases.* Traditionally, body fluids such as blood and urine are investigated in cases of intoxication and poisoning-related deaths; however, these samples are often no longer available for forensic toxicological analysis in decomposed and skeletonized corpses due to autolysis/putrefaction after burial and, possibly, time since death.[1,2] Substrates in postmortem toxicology are often seriously influenced by postmortem degradation, redistribution, matrix effects, temperature, etc. Therefore, interpretation of the results may be difficult.[3] In such cases, bones and visceral tissues that preserve structural integrity may be useful as alternative samples for toxicological analysis of toxic and drug poisoning cases.[4-6]

The goal of this experiment was to quantify drugs in putrefied/decomposed visceral tissues and buried bones and to determine the effect of burial on levels and distribution of drugs in tissues and bones collected from domestic pigs. Five *Sus scrofa domestica* pigs were used as research subjects. The experiment was conducted at the Experimental Research and Application Center of Medical Sciences in Cukurova University, Adana, Turkey. Drugs selected from various drug classes were divided into groups and administered to domestic pigs. The concentration of drugs was prepared to achieve an expected toxic level (for humans). Drugs were administrated to each pig, including a negative control, (n=4+1) respectively from pills (capsules and tablets), by gastrointestinal administration, and from solutions by intravenous administration. Peripheral blood, liver, cardiac muscle, spleen, kidney, brain, and bone samples from different anatomical locations (scapulae, humerus, and ulna from the upper extremity; cervical vertebrae, thoracal vertebrae, lumbar vertebrae, and rib from the thorax; ilium, femur, tibia, and fibula from the lower extremity) were collected from the pigs. The pigs were sacrificed four hours after administering propofol in order to allow for distribution and absorption of drugs. After organ samples were collected at 24, 48, 72, and 96 hours postmortem, bone samples were exhumed from corpses buried below soil ground at five and ten months. All samples were extracted using appropriate methods and analyzed by LC/MS/MS.

Only 9 of the 14 drugs were detected in the initial peripheral blood draw. A possible explanation why fentanyl, sertraline, tramadol, and zopiclone were not detected in initial blood draws may be the relatively small amounts that were administered, compared to the other drugs. According to this result, the drug levels in tissues were higher than in initial blood. For all bone types analyzed, the highest drug levels were detected from the thorax region and the lowest drug levels were detected from the lower extremities. Whereas the concentration of venlafaxine in fresh tibia was 0.94µg/g, it was 30.4µg/g in fresh ribs. It was observed that soft tissues liquefied when exhumation occurred at five months. Drug levels in bones collected at the fifth month increased. Whereas the concentration of venlafaxine in the tibia collected at the fifth month was 33.9µg/g, it was 94.3µg/g in the ribs. Given the fact that most of the soft tissue is located in the thorax region, it is possible that during the decomposition process, drugs partitioned from the liquefied tissue into bones. Drug levels detected in bones collected at the tenth month decreased.

These data demonstrated that drug levels in organ and bone samples collected from decomposed and buried corpses at different time intervals vary due to unknown mechanisms below ground where the corpse is exposed to the soil and completely unexplained conditions such as postmortem redistribution. These samples, collected under conditions in which blood is not appropriate for forensic toxicological analysis, are not uniformly useful for predicting blood concentration of drugs.

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Bone Analysis, Drugs Analysis, Exhumation

K27 Detection of Ketamine by Analyzing Dermestid Beetles Feeding on Buried, Dosed Rats by Liquid Chromatography With Tandem Mass Spectrometry (LC/MS/MS)

Thomas J. Nolan, BA, Arcadia University, 450 S Easton Road, Glenside, PA 19038; M. Lee Goff, PhD, Chaminade Univ of Honolulu, Forensic Sciences Program, 3140 Waialae Avenue, Honolulu, HI 96816-1578; Karen S. Scott, PhD, Arcadia University, 450 S Easton Road, Glenside, PA 19038; and Kimberlee S. Moran, MSc, Forensic Outreach, 231 Cedarbrook Road, Sicklerville, NJ 08081*

After attending this presentation, attendees will understand the method of extracting ketamine from dermestid beetles, larvae, pupae, shed molts, and frass using homogenization, liquid-liquid extraction, and an LC/MS/MS method.

This presentation will impact the forensic science community by creating a method for extracting and identifying the drug ketamine from dermestid beetles feeding on decomposing bodies. This presentation will also explain whether there is a dose-dependent relationship between the original dose of ketamine in the body and the concentration of ketamine in the dermestid beetles feeding on that body.

To date, there have been no studies conducted to investigate whether ketamine can be extracted and its presence identified from dermestid beetles feeding on dosed decomposing remains. In addition, there have been no studies investigating whether there is a dose-dependent relationship between original concentrations of ketamine in dosed buried bodies and dermestid beetles, which eat the decomposing remains.

The goal of this study is to determine the optimal method for homogenizing each stage of dermestid beetles' development, as well as shed molts and frass. This study also plans to discover the best extraction method for each of these samples and will use an LC/MS/MS method to identify the presence and quantity of ketamine in beetles feeding on dosed decomposed rats.

The rats were originally dosed at three ketamine concentrations: low, 20mg/kg (n=13); medium, 30mg/kg (n=13); and high, 40mg/kg (n=13). Beetles were also fed the remains of control rats that had been injected with saline and buried (n=4). One rat at each dosage (0, 20, 30, and 40) was left unburied and fed to dermestid beetles for comparative purposes. After dosage, the rats were euthanized using carbon dioxide. In addition, the effect of the decomposition stage on the detection of ketamine in dermestid beetles was investigated. The stages of decomposition were dug up at 77, 188, 293, 793, and 3,104 accumulated degree days. Eight adult dermestid beetles were placed into a container with the experimental decomposed rat remains to lay eggs. The adults do not eat the decomposed remains, but the larvae will as it grows. Once the beetles have eaten the entirety of the decomposed remains, the entire colony was frozen to kill the beetles. Each sample set was crushed using a mortar and pestle, and 300mg of each sample from each colony was collected for analysis. Ketamine was extracted from the samples using liquid-liquid extraction. The presence and quantity of ketamine and its metabolites, norketamine and dehydronorketamine, were found using LC/MS/MS with ketamine-D4 as the internal standard. It was found that the higher-dosed rats produced beetles with higher concentrations of ketamine; however, the concentrations were very variable and there does not appear to be a dose-dependent relationship for concentration of ketamine in the beetles.

Ketamine, LC/MS/MS, Entomotoxicology

K28 Determination of Zolpidem and Glyphosate in Blood From Emergency Room (ER) Patients

Hee-Sun Chung, PhD, Graduate School of Analytical Science and Tech, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 305-764, SOUTH KOREA*

After attending this presentation, attendees will be aware of the drugs and chemicals detected in ERs in Korea and the relationship between the blood level of the zolpidem and glyphosate and their symptoms in 15 zolpidem- and 6 glyphosate-positive cases.

This presentation will impact the forensic science community by showing how important it is to establish the screening method to identify the toxicants at ERs for proper treatment.

When intoxicated individuals are admitted to the ER, it is important to identify levels of intoxication and potential toxicants. Due to the absence of systematic analytical methods used in the ER at hospitals in South Korea, it is necessary to establish fast and accurate screening methods that can be readily used in the ER. In addition, it is important to evaluate the relationship between blood levels of potential toxicants and symptoms for the proper treatment of patients. The objectives of this study are: (1) to establish screening methods for identifying the chemicals in blood samples from patients; and, (2) to predict the clinical symptoms by comparing the relationship between blood levels of chemicals and clinical outcomes.

In this study, blood samples were collected from 80 patients who were admitted to Chungnam National University Hospital. Analytes of interest were isolated from each blood sample using Solid-Phase Extraction (SPE). These extracted samples were then analyzed using Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Mass Spectrometry (LC/MS). Method validation was performed for the most commonly encountered compounds (zolpidem and glyphosate), including linearity, Limit Of Detection (LOD), Limit Of Quantitation (LOQ), intra- and inter-day precision, and accuracy. Clinical symptoms and Glasgow Coma Scale (GCS) scores were also recorded at the ER. Measured GCS scores ranged from 3 (full coma state) to 15 (full alert).

As a summary of results, a variety of compounds were identified from the 80 blood samples analyzed in this study, including zolpidem (*n*=15), diphenhydramine (*n*=9), tramadol (*n*=8), acetaminophen (*n*=8), chlorpheniramine (*n*=6), quetiapine (*n*=5), glyphosate (*n*=5), and imipramine (*n*=3). Blood levels of zolpidem in 15 cases and glyphosate in 5 cases ranged from 19.6ng/mL to 3,605.8ng/mL and from 24.3ng/mL to 165.4 ng/mL, respectively. The mental state of patients intoxicated with these two compounds was alert to semi-coma with GCS scores of 3 (eye 1, verbal 1, motor 1) to 15 (eye 4, verbal 5, motor 6).

In conclusion, it is demonstrated that blood levels of zolpidem and glyphosate were well correlated with clinical symptoms and GCS scores from patients. Therefore, developed methods using GC/MS and LC/MS with SPE can be utilized as screening tools to determine the level of intoxication and type of toxicants. In addition, the evaluated information will be useful for clinical toxicologists in order to provide the appropriate treatment of patients in the ER.

ER Patient, Zolpidem, Glyphosate

K29 Quetiapine Stability as It Relates to the Time Frames of Case Studies

Mariah D. Carson, BS, Sam Houston State University, 124 Bolero Way, Huntsville, TX 77340; and Jeffrey Walterscheid, PhD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054*

WITHDRAWN

K30 Mass Spectral Library for Phosphodiesterase Type 5 Inhibitors by Ultra High-Performance Liquid Chromatography/Quadrupole-Time-of-Flight/Mass Spectrometry (UHPLC/Q-TOF/MS)

Marissa J. Finkelstein, MS, Miami-Dade Medical Examiner Department, 1851 NW 10th Avenue, Forensic Toxicology, Miami, FL 33136; Mathew Hautman, BS, Aegis Sciences Corporation, 365 Great Circle Road, Nashville, TN 37228; Lucas Marshall, MS, Aegis Sciences Corporation, 365 Great Circle Road, Nashville, TN 37228; Rebecca Heltsley, PhD, 515 Great Circle Road, Nashville, TN 37228; Timothy A. Robert, PhD, 515 Great Circle Road, Nashville, TN 37228; and David L. Black, PhD, Aegis Sciences Corporation, 515 Great Circle Road, Nashville, TN 37228*

After attending this presentation, attendees will understand the process of creating and verifying a mass spectral library for Phosphodiesterase Type 5 (PDE5) enzyme inhibitors. In addition, attendees will better understand the analysis of these compounds in dietary supplements.

This presentation will impact the forensic science community by demonstrating the utility of mass spectral library searching for the detection of PDE5 inhibitors in dietary supplements.

According to recent statistics, PDE5 inhibitors were the most common adulterant detected in the Food and Drug Administration (FDA) -recalled dietary supplements from January 2004 through December 2012. PDE5 inhibitors such as sildenafil (Viagra®), vardenafil (Levitra®), and tadalafil (Cialis®) are commonly prescribed for treatment of Erectile Dysfunction (ED); however, when included in dietary supplements as an off-label ingredient, PDE5 inhibitors will cause serious drug-drug interactions for men taking certain heart medications. In competitive sports, athletes may use PDE5 inhibitors for their potential performance-enhancing effects. PDE5 inhibitors are vasodilators and thus may allow for excess blood flow and oxygenation. These compounds are not currently banned by the World Anti-Doping Agency, though there is some debate as to whether they would provide an unfair advantage to athletes who compete at higher altitudes or in long-distance sporting events such as cycling.

The objective of this study was to utilize UHPLC/qTOF/MS to develop a screening method for PDE5 inhibitors based on mass accuracy and mass spectrum library searching.

To build the library, mass spectra were obtained by injecting 1.0µg/mL neat reference standards in Information Dependent Acquisition (IDA) mode. The mass spectral data was analyzed utilizing PeakView® and MasterView® software. The mass spectral library was created using LibraryView® software. Extraction efficiencies of fortified PDE5 inhibitors were compared between Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) and methanolic extractions of two dietary supplements, a powdered hydration supplement and an oral form of hyaluronic acid. The UHPLC/qTOF/MS utilized reversed phase separation operated in positive electrospray ionization mode.

Nine analytes were included in the PDE5 inhibitor library and all successfully triggered a library match at100ng/mL or greater for fortified extracted samples. The results were scored based on mass error, retention time error, isotope ratio error, and library match; an evaluation of the combined scores showed a threshold of at least 80 to be considered a non-negative sample. Regardless of extraction procedure or dietary supplement matrix investigated, all analytes produced mass spectral library matches and showed a combined score ranging from 84.8 to 98.2. The instrumental limit of detection was analyzed by injecting neat reference standards of each analyte at concentrations ranging from 5ng/mL to 500ng/mL. The retention time and mass accuracy show that all of the analytes are detectable as low as 5ng/mL, but Information Dependent Acquisition (IDA) product ion scans were not triggered for all of the analytes until the concentration reached 100ng/mL.

Fortified matrix samples and four FDA recalled over-the-counter male enhancement supplements were analyzed to verify the utility of the library. The dietary supplement matrices were fortified from 25 to 200ng/mL with a PDE5 inhibitor standard mix. For samples fortified at 200ng/mL, library matches were successfully produced for all nine analytes in the PDE5 inhibitor mix. The extraction of the FDA-recalled supplements found the active pharmaceutical ingredients sildenafil (Viagra®) and tadalafil (Cialis®) in all four supplements.

A mass spectral library was successfully developed for PDE5 inhibitors and verified by both methanol and QuEChERS extractions as low as 100ng/mL for the supplements tested. The method was challenged by extracting two different fortified dietary supplements and four FDA-recalled, over-the-counter male enhancement supplements; it was effective at identifying PDE5 inhibitors in these matrices.

PDE5 Inhibitors, LC/qTOF/MS/MS, Dietary Supplements

K31 Effective Extraction Strategies for Buprenorphine and Norbuprenorphine in Urine, Oral Fluid, and Whole Blood Using Cation Exchange Solid Phase Extraction (SPE) and Supported Liquid Extraction (SLE) Prior to High-Performance Liquid Chromatography With Tandem Mass Spectrometry (HPLC/MS/MS) Analysis

Victor Vandell, PhD, Biotage, 10430 Harris Oaks Boulevard, Charlotte, NC 28269; Elena Gairloch, BS, Biotage, 10430 Harris Oaks Boulevard, Charlotte, NC 28269; and Bruce R. Kempf, BS, Biotage, 10430 Harris Oaks Boulevard, Charlotte, NC 28269*

After attending this presentation, attendees will have seen a sample preparation, method development, and results achieved for target analytes in specific matrices using SPE and SLE prior to LC/MS/MS analysis.

This presentation will impact the forensic science community by showing how analyte stability issues during sample preparation of buprenorphine and norbuprenorphine can be overcome using a fast and reliable SLE or SPE extraction followed by LC/MS/MS analysis.

Introduction: Buprenorphine and norbuprenorphine are typically problematic for analysis due to analyte stability issues during sample preparation. A fast and reliable testing protocol is needed to address extracting the target analytes out of complex matrices typically encountered during toxicological testing. A fast, reliable, and robust sample preparation method that could be implemented to extract these drugs from complex biological matrices with good analyte recovery and minimum matrix effects would be ideal for toxicology laboratories. New, rapid, and reliable sample preparation methods used to extract the target analytes from small amounts of biological matrix were demonstrated. These methods were fully automated on the Biotage® Extrahara® Sample Preparation Workstation. Qualitative and quantitative data demonstrates the utility of these methods prior to LC/MS/MS analysis.

Method: SLE in a 96-fixed well plate format was used to extract buprenorphine and norbuprenorphine from whole blood spiked at concentrations from 1.0 ng/mL to 100ng/mL. Sample pre-treatment consisted of a 1:3 dilution of blood (100µL) with 0.1% ammonium hydroxide (300µL). An optimal extraction solvent of ethyl acetate:acetonitrile:ammonium hydroxide was identified. Cation exchange SPE in a 96-well plate format was employed for the extraction of the same target analytes from urine and oral fluid (neat and buffered) spiked at a concentration range of 0.1ng/mL-100ng/mL. Sample preparation consisted of a 1:9 dilution of the matrix with 0.1% formic acid. Both extraction processes were fully automated on the Biotage® Extrahera® Sample Preparation Workstation. Extracts were evaporated to dryness, re-constituted in mobile phase, and injected onto an Agilent® 1200 coupled to a SCIEX™ 4000 Q-TRAP® triple quadrupole mass spectrometer for analysis.

Results: Averaged recoveries of greater than 90% were observed for the target analytes in urine and oral fluid. Averaged recoveries greater than 70% were observed for analytes in whole blood. All averaged recovery percent Relative Standard Deviations (% RSDs) were calculated at less than 10%. Measured matrix effects ranged from 8% to 50% for the analytes in all three matrices. In-house calibrators were prepared in urine, blood, and oral fluid matrices. Calibration curves were generated for the analytes across the dynamic range of 0.1ng/ml to 100ng/ml for samples in oral fluid and urine. Calibration curves were generated across the dynamic range of 1.0ng/ml to 100ng/ml for the analytes extracted from whole blood. All calibration curves were linear with r^2 >0.99. The precision and accuracy of the in-house calibrators was determined to be within ±15%. The limit of detection for all three matrices was determined to be 0.1ng/ml and the Limit Of Quantitation (LOQ) for the analytes in urine and oral fluid was determined to be 0.2ng/ml. The LOQ for the analytes in whole blood was determined to be 0.5ng/ml.

Conclusion: This study presents a fast, simplified approach for extraction of buprenorphine and norbuprenorphine with reproducible recoveries for low analyte detection levels (0.1ng/ml) using a minimal amount of sample (100µL).

Sample Prep, Drugs, LC/MS/MS

K32 Quantification of Buprenorphine and Norbuprenorphine in Postmortem Blood and Urine by Ultra High-Performance Liquid Chromatography/Tandem Mass Spectrometry (UHPLC/MS/MS)

*Chu-An Yang, MS**, No 123, Min'an Stre, Zhonghe District, New Taipei City 235, TAIWAN, ROC; Hsiu-Chuan Liu, MS, Taipei, Taipei, TAIWAN, ROC; Ray H. Liu, PhD, 12512 NW 50th Drive, Vancouver, WA 98685; and Dong-Liang Lin, PhD, Institute of Forensic Medicine, 123, Min-An Street, Zhonghe District, New Taipei City 23548, TAIWAN, ROC*

After attending this presentation, attendees will gain insight into a highly sensitive UHPLC/MS/MS approach for the quantifications of buprenorphine and norbuprenorphine in blood and urine specimens that have been prepared by a liquid-liquid extraction process.

This presentation will impact the forensic science community by describing how the development and validation of the UHPLC/MS/MS method will improve forensic laboratories' abilities in the quantification of buprenorphine and norbuprenorphine in postmortem specimens.

Buprenorphine is prescribed for patients in heroin treatment programs in Taiwan. It is also used for treating moderate to severe chronic pain. In humans, buprenorphine is metabolized to norbuprenorphine by N-dealkylation. The purpose of this study was to develop an effective UHPLC/MS/MS-based methodology that is simple, accurate, and sensitive for the quantification of buprenorphine and norbuprenorphine in blood and urine at low concentration levels.

Blood or urine (1mL) were mixed with sodium carbonate/bicarbonate buffer (pH=9.5) and extracted with ethyl acetate. Extracts were evaporated and reconstituted in the mobile phase (initial gradient composition) for injection onto the UHPLC/MS/MS system. Deuterated analogues of the analytes were used as internal standards. Chromatographic separation was achieved using an Agilent® ZORBAX® SB-Aq (100mm ´ 2.1mm i.d., 1.8-µm particle) analytical column at 50oC. The mobile phase included 0.1% formic acid (v/v) in water (A) and methanol (B), with a flow rate of 0.32mL/min. The initial gradient composition (A/B 90:10, v/v) was held for 1.5min; decreased to 0% A in 10min and held for 2min, then increased to 90% A in 1min and held for 2min. Parameters for mass spectrometric analysis included: (1) Agilent®Jet Stream technology electrospray ionization in positive-ion Multiple Reaction Monitoring (MRM) mode; (2) optimized collision energy levels for selected precursor ions; and, (3) monitoring two or three transitions for analytes and internal standards.

Method validation was performed using drug-free blood and urine that were fortified with 1ng/mL-20ng/mL of the analytes. The following analytical parameters were obtained: (1) average extraction recovery, derived from four different sources of blood and urine, was higher than 60%; (2) matrix effect (ion enhancement) was observed, except for urine samples at the 10ng/mL and 20ng/mL concentration levels, but was adequately compensated for by respective deuterated internal standards; (3) intra-/inter-day precision (%CV) and accuracy ranges for blood were 0.45%-8.6% / 1.7%-10% and 95%-108% / 97%-105%, while the corresponding ranges for urine were 0.49%-4.1% / 2.0%-7.9% and 96%-107% / 94%-113%; and, (4) calibration linearity (r^2) for both analytes were >0.997; the limits of detection and quantification for buprenorphine and norbuprenorphine were 0.01ng/mL and 0.025ng/mL (urine) and 0.075ng/mL and 0.075ng/mL (blood), respectively. When applied to case samples, postmortem urine specimens were first hydrolyzed by an enzymatic method prior to the extraction step. In conclusion, this relatively simple protocol was found to be effective and reliable for routine identification and quantification of buprenorphine and norbuprenorphine in blood and urine. This method was successfully applied to the analysis of postmortem and antemortem specimens from forensic cases.

Buprenorphine, Postmortem, UHPLC/MS/MS

K33 The Analysis of N,N-Dimethyltryptamine (DMT) in Plasma by Liquid Chromatography/ Tandem Mass Spectrometry (LC/MS/MS)

David M. Andrenyak, PhD, University of Utah, Center for Human Toxicology, 30 S 2000 E, Rm 105, Salt Lake City, UT 84112; and David E. Moody, PhD, Center for Human Toxicology, University of Utah, 30 S 2000 E, Rm 105, Salt Lake City, UT 84108*

After attending this presentation, attendees will better understand, through actual demonstration, an LC/MS/MS method to analyze DMT in plasma.

Hallucinogenic drugs such as DMT are popular drugs of abuse. It is important that there are effective analytical methods to test for these drugs in biological samples such as plasma. This presentation will impact the forensic science community by illustrating how to analyze for DMT in plasma by LC/MS/MS.

DMT is an indole compound that produces hallucinogenic effects. It is a **Drug Enforcement Administration** (DEA) Schedule I controlled substance. DMT is found in South American hallucinogenic plants such as *Piptadenia peregrina*. DMT is structurally similar to the neurotransmitter serotonin and may exert it effects on neuro-mechanisms that involve serotonin. Small amounts of endogenous DMT (generally less than 1ng/mL) have been detected in human plasma, but typical DMT use results in plasma concentrations in the 10ng/mL to 100ng/mL range. A method utilizing LC/MS/MS was developed to determine DMT in rat plasma samples. An effort to achieve a 1.0ng/mL DMT Limit Of Quantitation (LOQ) for the analysis seemed reasonable.

For the analysis, a 0.1mL volume of calibrators (range 1ng/mL to 500ng/mL) and controls (3ng/mL, 25ng/mL, and 400ng/mL) were prepared in separate, clean 16mm x 100mm culture tubes. Each tube was fortified with 30ng/mL of 5-methyl-DMT (30µL of 0.1ng/µL) as the internal standard. Liquid-liquid extraction was used to prepare the samples for analysis: 0.5mL water, 0.1ml ammonium hydroxide, and 3mL of 1-chlorobutane:acetonitrile (4:1) were added to each tube. The tubes were mixed for 20 minutes on a reciprocating shaker and centrifuged. The organic layer from each tube was collected into separate, clean 13mm x 100mm glass culture tubes. A 0.1mL volume of 0.1% hydrochloric acid in methanol was added to each extract tube. The extracts were evaporated to dryness using a Turbovap® evaporator, reconstituted with 0.2mL of 10mM ammonium acetate, pH 5:methanol (85:15), and transferred to separate conical polypropylene autosampler vials. The LC/MS-MS analysis used an Agilent® 1100 LC interfaced with an Access TSQ Quantum® MS and was operated by Xcalibur™ version 2.0 SR2 software. The LC conditions involved the use of a Selectra® DA 100 x 2.1mm, 3µm column. The isocratic mobile phase was 10mM ammonium acetate, pH 5.0:methanol (55:45) at a 0.2mL/minute flow rate. The MS/MS analysis employed positive ion electrospray ionization and Selected Reaction Monitoring (SRM). For each compound, the precursor ion was the protonated molecular ion. The SRM transitions that were monitored were: DMT: 189®144 (quant.), 189®58; 5-methyl-DMT: 203®158. The DMT and 5-methyl-DMT were chromatographically separated with good peak shape. Ion suppression evaluation showed that blank plasma extracts did not suppress the MS/MS signal for DMT and 5-methyl-DMT. For six different blank plasma sources, blank matrix selectivity studies showed that peak area ratios from endogenous compounds in blank plasma were well below 20% of the 1ng/mL DMT (LOQ) peak area ratio. Precision and accuracy were evaluated by analyzing the control samples. The intra-run accuracy ranged from 90.3% to 106.9% of target and the intra-run precision ranged from 1.7% to 3.7%. The inter-run accuracy ranged from 101.8% to 113.0% of target and precision ranged from 4.6% to 10.4%. The extracts were stable for at least six days at room temperature and 19 days at 4°C. In conclusion, this study developed an LC/MS/MS method that was capable of analyzing for DMT in rat plasma. Cross-validation to human plasma is planned.

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N,N-Dimethyltryptamine, LC/MS/MS, Analysis

K34 Rapid Screening and Quantitation of Pesticides in Biological Samples Using Gas Chromatography (GC) With Mass Spectrometer (MS)

Muhammad Taimoor Chaudhary, MPhil, Punjab Forensic Science Agency, Thokar Niaz Baig Multan Road, Lahore, Punjab, PAKISTAN; and Mohammad A. Tahir, PhD, Punjab Forensic Science Agency, Thokar Niaz Beig, Multan Road, Lahore, PAKISTAN*

After attending this presentation, attendees will better understand not only the ongoing potential of GC coupled with MS in the detection of common pesticides along with a suitable single step Liquid-Liquid Extraction (LLE) procedure, but will also better understand the current misuse of different types of pesticides used in Pakistan.

This presentation will impact the forensic science community by providing results of case studies regarding the use of pesticides for suicidal and homicidal purposes. Furthermore, a rapid GC/MS run (in seven minutes), Selected Ion Monitoring (SIM), and scan mode, for the analysis of common pesticides, has not been reported earlier. This information will be highly appreciated in clinical and forensic settings.

Pesticides are a common source of oral and inhaled poisoning in Pakistan. Cases have been reported regarding accidental and/or intentional poisoning of some commonly used pesticides. Rapid identification of pesticides in biological samples is not only necessary for early treatment decisions in clinical settings but also important for the forensic toxicology cases.[1] Analytical methods devised for the determination of pesticides in food and plant items are not directly useful for biological matrices as sample cleanup is a major hindrance.[2-10] Despite the benefits of Solid Phase Extraction (SPE), Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS), pressurized liquid extraction, and Solid Phase Microextraction (SPME), LLE is still the most commonly used extraction technique of the past two decades.[11]

Basified postmortem samples (2mL) were extracted in a single step by using ethyl acetate:dichloromethane (5mL of 1:1v/v). All extracts were reconstituted with ethyl acetate and hexane in (1:1 v/v), run on a gas chromatograph with DB-5ms capillary column and detected by using an MS on both SIM and scan mode, simultaneously, in a run time of seven minutes.

The method was validated for various pesticides (carbofurane, atrazine, chlorpyrifos, buprofezin, bifenthrin, pyriproxyfen, lambda-cyhalothrin, cypermethrin, and deltamethrin) by using spiked synthetic blood samples with five point calibration (0.50mg/L-3.75mg/L). Limit Of Detection (LOD) ranged from 0.202mg/L-0.657mg/L, while the Limit Of Quantitation (LOQ) varied from 0.50mg/L-1.00mg/L. Accuracy (82.0%-115.20%) and precision (as %CV; 2.14%-17.50%) at three different concentration levels (1.0mg/L, 2.5mg/L, and 3.75mg/L) were within acceptable range (80%-120% and <20%, respectively). Postmortem samples of homicide and suicide cases, submitted to the laboratory, were analyzed for the presence and quantitation of pesticides. Various cases were found to be positive for the stated pesticides in whole blood, gastric contents, and liver tissues.

The objective of this work was to screen and quantitate some common pesticides from postmortem cases of homicides and suicides. GC/MS (electron impact ionization) is a rapid and sensitive technique for the measurement of pesticides in different forensic toxicology samples.

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K35 Applicability of Biochip Array Technology to the Simultaneous Screening of Drugs Associated With Driving Under the Influence of Drugs (DUID)

Gemma Mullan, PhD, 55 Diamond Road, Crumlin, UNITED KINGDOM; William Snelling, MS, Randox Toxicology Limited, 55 Diamond Road, Crumlin, UNITED KINGDOM; Laura Keery, BSc, Randox Toxicology Limited, 55 Diamond Road, Crumlin, UNITED KINGDOM; Joanne Darragh, PhD, 515 Industrial Boulevard, Kearneysville, VA 25430; Pankaj Sinha, 515 Industrial Boulevard, Kearneysville, VA 25430; Maria Luz Rodriguez, PhD, Randox Toxicology Limited, 55 Diamond Road, Crumlin, UNITED KINGDOM; R. Ivan McConnell, BSc, 515 Industrial Boulevard, Kearneysville, VA 25430; and S. Peter Fitzgerald, PhD, Randox Laboratories, Ltd, 55 Diamond Road, Crumlin BT29 4QY, UNITED KINGDOM*

After attending this presentation, attendees will better understand the application of biochip array technology to the simultaneous screening of drugs associated with DUID, which are under-reported recommendations focused on a two-tier approach of drug analysis.

This presentation will impact the forensic science community by providing results from a new biochip array that allows simultaneous determination of drugs associated with DUID and included in Tier 1 and Tier 2 under reported recommendations. Twenty immunoassays arrayed on the biochip surface allow this multi-analytical screening from a single whole blood sample. This leads to test consolidation and an increase in the screening capacity in test settings.

Biochip array technology enables the simultaneous detection of multiple analytes from a single sample. As drug impaired driving is becoming a major problem in the United States and worldwide, recommendations for the toxicological investigation of drug-impaired driving and motor vehicle fatalities were reported. These recommendations focused on a two-tier approach of drug analysis. Tier 1 consisted of the most prevalent drugs found in the United States impaired-driving population with Tier 2 drugs being less frequently encountered, with regional significance and/or beyond the routine analytical capabilities of some laboratories. Tier 1 drugs should be the minimum testing that should be completed in drugged driving casework.[1]

Competitive chemiluminescent biochip-based immunoassays were employed. Ligands were immobilized and stabilized to the biochip surface defining an array of 20 discrete test sites (15 Tier 1 assays and 5 Tier 2 assays). The signal output is inversely proportional to the concentration of the drug in the sample.

Tier 1 assays included were: Amphetamine (AMPH), Methamphetamine (MAMP), Barbiturate (BARB), Benzodiazepine Class 1 (BENZ1), Benzodiazepine Class 2 (BENZ2), Cannabinoids (THC), Cocaine/Benzoylecgonine (BZG), Hydromorphone (OPDS), Meprobamate (MPB), Methadone (MDONE), Opiates (OPIAT), Oxycodone (OXYC1 and OXYC2), Phencyclidine (PCP), and Zolpidem (ZOL). Tier 2 assays included: Buprenorphine (BUP), Dextromethorphan (DMP), Fentanyl (FENT), Tramadol (TRM), and Tricyclic Antidepressants (TCAs). The assays are semi-quantitative and applicable to both the fully automated Evidence Analyser and the semi-automated analyzer Evidence Investigator. The systems have dedicated software to process, report, and archive the data produced. The sample volume required is 60µl of whole blood (diluted one in four).

In this initial evaluation, the cut-offs of all the assays were within the values stated in the recommendations. The assays presented the following Limits Of Detection (LOD): for Tier 1 drugs — AMPH 4.35ng/mL, MAMP 2.44ng/mL, BARB 4.41ng/mL, BENZ1 0.26ng/mL, BENZ2 0.83ng/mL, THC 1.78ng/mL, BZG 1.31ng/mL, OPDS 1.37ng/mL, MPB 13.15ng/mL, MDONE 0.61ng/mL, OPIAT 0.48ng/mL, OXYC1 0.87ng/mL, OXYC2 2.22ng/mL, PCP 0.10ng/mL, and ZOL 0.45ng/mL; for Tier 2 drugs assays presented the following LODs — BUP 0.06ng/mL, DMP 0.3ng/mL, FENT 0.13ng/mL, TRM 0.53ng/mL, and TCA 1.98ng/mL. Intra-assay precision around the cut-off value for each of the assays, expressed as %CV (*n*=6), ranged between 5.5% and 16.3%.

In conclusion, the results indicate applicability of biochip array technology to the simultaneous screening of drugs associated with DUID and included in Tier 1 and Tier 2 drugs under reported recommendations. The 20 immunoassays arrayed on the biochip surface presented lower LODs than the recommended cut-offs in whole blood. This methodology allows for multi-analytical screening of samples, leading to test consolidation and increased screening capacity in test settings.

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DUID, Biochip Array, Tier 1

K36 Fragmentation Pathways and Structural Characterization of Mitragynine and Its Metabolite Using Electrospray Ionization (ESI) and High Resolution Mass Spectrometry

Stephanie Basiliere, BS, 3019 Sam Houston Avenue, Apt G302-2, Huntsville, TX 77340; Sarah Kerrigan, PhD, Sam Houston State University, 1003 Bowers Boulevard, SHSU Box 2525, Huntsville, TX 77341; and Kelsie Bryand, MS, PO Box 2525, Huntsville, TX 77340*

After attending this presentation, attendees will be able to identify common fragmentation pathways associated with Mitragynine (MG), 7-Hydroxymitragynine (OH), and other corynanthe-type alkaloids.

This presentation will impact the forensic science community by highlighting the importance of structural identification during routine method development.

MG (9-methoxycorynantheidine, Kratom) is a naturally occurring corynanthe-type indole alkaloid that is present in the leaves of *Mitragyna speciosa*. This flowering plant of the *Rubiaceae* genus contains more than 20 alkaloids, of which MG is the principal pharmacologically active component. While OH is a minor constituent, it is considerably more potent and is also produced as a metabolite following MG use. Although MG is structurally related to yohimbine, its effects are notably different. Unlike yohimbine, which has pronounced adrenergic and serotonergic effects, MG behaves as a μ-opioid receptor agonist. While not federally regulated in the United States, Kratom's dual stimulant and opiate-like effects are somewhat unique, making it an ideal candidate for misuse among recreational drug users.

Although Gas Chromatography/Mass Spectrometry (GC/MS) is the most widely used technique in forensic toxicology laboratories, identification of MG-OH in biological samples presents a significant challenge in terms of analytical detection. Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS) is a high resolution MS technique that offers high sensitivity and significant benefits in terms of mass accuracy and structural identification. Tandem Mass Spectrometry (MS/MS) spectra following optimization of ionization conditions can provide valuable structural information. Characterization of fragmentation pathways and subsequent structural identification of ions should play an important role in new assay development.

During the development of an analytical method to determine MG and MG-OH in urine using ESI/LC/qTOF/MS, the fragmentation pathways for MG, OH, and their deuterated analogs were investigated. MS/MS spectra were used to tentatively identify fragments and make mass assignments. Ultimately, this process plays an important role in the selection of highly specific precursor ion transitions. A total of three transitions were selected for each of the compounds (and their respective internal standards).

The most abundant product ions for both MG and MG-OH were associated with C-ring cleavage and the loss of the substituted piperidine (D-ring) between C2 and C5. The abundance and specificity ultimately led to this being selected for quantitation for both MG (399→174) and MG-OH (415→190). Variations of C-ring cleavage predominated for all other major product ions, as well as formation of intact substituted piperidine ions.

Chromatographic separation and mass spectral acquisition are particularly important analytical variables due to the potentially large number of structurally similar alkaloids and diastereoisomers found in *M. speciosa*. LC/qTOF/MS and other high resolution MS techniques are particularly useful for complex analytes such as these.

Mitragynine, 7-Hydroxymitragynine, Fragmentation

K37 *In Vitro* Metabolism Studies on P-Methoxyamphetamines (PMA) Using Human Liver Microsomes and Liquid Chromatography With Tandem Mass Spectrometry (LC/MS/MS) With Chemical Derivatization

Tanasiri Yokchue, MSc, Forensic Medicine & Science, University of Glasgow, Joseph Black Building, University Place, Glasgow G12 8QQ, UNITED KINGDOM; and Robert A. Anderson, PhD, Forensic Medicine and Science, University of Glasgow, Glasgow G12 8QQ, UNITED KINGDOM*

After attending this presentation, attendees will better understand PMA and its metabolism, the practice and utility of *in vitro* metabolic studies using Human Liver Microsomes (HLM), the usefulness of chemical derivatization in Liquid Chromatography/ Mass Spectrometry (LC/MS), and the identification of drug metabolites by conventional LC/MS/MS.

This presentation will impact the forensic science community by providing results from *in vitro* studies in an area with very little previous research and will broaden the understanding of how *in vitro* metabolism by HLM can overcome the problems of lack of reference standards, human metabolic studies of new drugs, and how to identify metabolites by LC/MS.

PMA is a toxic mono-methoxy amphetamine derivative with strong stimulant and hallucinogenic properties which has been implicated in deaths since the 1970s.[1,2] The few metabolism studies of PMA that have been published have shown that the major pathway is O-demethylation to 4-Hydroxyamphetamine (PHA), which is partially conjugated. A minor metabolic pathway is beta-hydroxylation to β-hydroxy-PMA, followed by O-demethylation to 4-hydroxynorephedrine.[3]

The forensic toxicologist faces significant problems in the analysis of novel psychoactive substances such as PMA, both because of lack of information on their metabolism and because of the unavailability of reference standards of the drugs and their metabolites. Ethical considerations usually preclude human metabolism studies under controlled conditions and *in vitro* methods provide potential alternatives. An additional challenge is the analysis of these polar metabolites using conventional C18 High-Performance Liquid Chromatography (HPLC) columns, resulting in low retention volumes. One rapid and simple solution to this is conversion of metabolites to more hydrophobic compounds by chemical derivatization, to improve separations by reversed-phase HPLC and increase sensitivity in MS.

The goal of this study was to identify the metabolites of PMA in humans using HLM *in vitro* and reversed-phase LC/MS/MS with derivatization.

Pooled human liver microsomes were incubated with NADPH regenerating system and PMA in 0.1M phosphate buffer pH 7.4 in a shaking incubator at 37oC for 90 minutes. Each reaction was stopped by the addition of ice-cold acetonitrile and extracts were evaporated and derivatized with acetic anhydride/pyridine (3:2 v:v) for 30 minutes at 60oC. Derivatized metabolites were stable in mobile phase for up to 30h and were separated on a conventional C-18 column with retention (capacity) factors in the range 1.55-2.94.

Three phase-I metabolites (both major and minor metabolites) of PMA were identified by LC/MS/MS using Multiple Reaction Monitoring (MRM). PMA derivative (PMA-Ac) was identified by MRM transitions at m/z 208>121, 208>149, and 208>91. A major metabolite, P-Hydroxyamphetamine Derivative (PHA-2Ac), was identified by MRM transitions at m/z 236>194, 236>135, and 236>107. A minor metabolite, β-Hydroxy-Metabolite (β-OH-PMA-2Ac) was identified by MRM transitions at m/z 266.3>224, 266.3>165, and 266.3>91 and 4-Hydroxy-3-Methoxyamphetamine Derivative (HMA-2Ac) was identified by MRM transitions at m/z 266>165, 266>137, and 266>105.

In conclusion, HLM can be used to simulate drug metabolism in humans and to provide chromatographic and mass spectrometric data on metabolites. Acetate derivatives result in higher molecular weights, providing more specific ions for identification, and in decreased polarities of metabolites, improving their analysis on reversed-phase C18 columns. This method would be suitable for routine analysis of urine to detect and confirm abuse of PMA.

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PMA, *In Vitro* Metabolism, LC/MS

K38 Analysis of Illicit Substances in Urine by Biocompatible Solid-Phase Microextraction (BioSPME) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Kaitlyn E. Hess, BS, Cedar Crest College, 100 College Drive, Allentown, PA 18104; and Thomas A. Brettell, PhD, Cedar Crest College, 100 College Drive, Allentown, PA 18104*

After attending this presentation, attendees will have a better understanding of how BioSPME fibers can work as a purification step in extracting drug analytes from biological fluids prior to analysis by LC/MS/MS.

This presentation will impact the forensic science community by providing a simpler and faster extraction alternative to preparing biological samples for analysis using an LC/MS/MS method that is able to simultaneously detect and differentiate illicit substances from varying classes of drugs.

Forensic toxicologists are faced with growing case workloads and demands to produce accurate results within short periods of time. What is even more challenging is the speed at which new substances are being introduced to the black market. New drugs, though they may be similar in structure to commonly recognized drugs, can evade detection by specific techniques like immunoassays, making them difficult or nearly impossible to identify. Moreover, because of the rise in synthetic drug production, crime laboratories face a large backlog and challenge in identifying these illicit substances in a timely and efficient manner using the current extraction procedures.

LC/MS/MS is commonly used by forensic toxicologists for the detection of drug analytes in biofluids. Furthermore, SPME has been used as a solventless purification step prior to LC/MS/MS to avoid complicated or timely extraction procedures. A new BioSPME fiber has been engineered and is being explored for its use in extracting drug analytes from different biological matrices. The BioSPME fiber is stationed within a pipet tippet and is functional in a 96-well format. Each fiber contains either mixed-mode hydrophobic and cation exchange particles or C-18 (reversed-phase) particles to extract drug analytes of interest. The fiber is directly placed into a biological fluid (urine) for extraction, desorbed into solution, dried, and reconstituted for analysis by LC/MS/MS. The method presented here utilized an AB SCIEX™ 3200 Qtrap® triple quadrupole mass spectrometer interfaced with a Shimadzu® LC system. Extracted samples were run in positive-ionization mode using Electrospray Ionization (ESI). Chromatography was performed using an Ultra C18 column (50 x 2.1mm, 3µm). The weak mobile phase was 0.1% (v/v) formic acid in water and the strong mobile phase was 0.1% (v/v) formic acid in acetonitrile. A gradient curve was used over a run time of five minutes per sample. The flow rate was 0.4mL/min., the column temperature was set at 30° Celsius, and the injection volume was 5µL. Drugs from different classes were studied for their extraction efficiency from urine with this novel BioSPME method using both mixed-mode and C-18 BioSPME fiber chemistries. Extraction variables such as pH and extraction time, desorption time and extraction solvents, drying time, and reconstitution solvents were considered in this study.

Seven drugs have been successfully extracted. Extraction recoveries for alprazolam, amphetamine, methamphetamine, cocaine, benzoylecgonine, mephedrone, and MDPV are as follows: 54.4%, 17.8%, 40%, 60%, 18.2%, 41.5%, and 49.5%, respectively. Limit of detection and limit of quantitation data for the same drugs were: 6.5ng/mL and 19.3ng/mL, 12.0ng/mL and 36.5ng/mL, 5.4ng/mL and 16.3ng/mL, 21.5ng/mL and 65.2ng/mL, 7.9ng/mL and 24.0ng/mL, 4.1ng/mL and 12.4ng/mL, and 6.4ng/mL and 19.5ng/mL, respectively. Extraction efficiency ranged from 17% to 60% for the drugs tested.

By utilizing BioSPME's direct sampling capabilities, time-consuming extraction procedures that were previously needed to free drug analytes from biological matrices can be eliminated. Extracted substances are differentiable from one another when analyzed by LC/MS/MS; thus, this method can be used to simultaneously detect multiple substances from a biological matrix. In turn, this method may be used to decrease the burden of lengthy extraction procedures in forensic toxicology laboratories.

Forensic Toxicology, BioSPME, LC/MS/MS

K39 Evaluation of the Components Within Electronic Cigarette Liquids and Drugs of Abuse Using Gas Chromatography/Mass Spectrometry (GC/MS) and Ultra-Fast Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)

Erin Walsh, Boston University School of Medicine, 72 E Concord Street, L-805C, Boston, MA 02118; Robert D. Johnson, PhD, Tarrant County MEO, 200 Feliks Gwozdz Place, Fort Worth, TX 76104; Peter Tracy, BS, Suffolk County Crime Laboratory, 725 Veterans Memorial Highway, Hauppauge, NY 11788; and Sabra R. Botch-Jones, MS, MA, Boston University School of Medicine, Biomedical Forensic Sciences, 72 E Concord Street, Boston, MA 02118*

After attending this presentation, attendees will have a better understanding of electronic cigarettes, electronic cigarette liquids, the growing trend of using these products to abuse drugs, and how to analyze electronic cigarette liquids suspected of containing drugs of abuse.

This presentation will impact the forensic science community by demonstrating that detection and quantification of drugs of abuse added to electronic cigarette liquids is possible and by describing the effect that the electronic cigarette liquid matrix has on these results.

As electronic cigarettes become more prevalent in society, their use as a delivery mechanism for drugs of abuse has increased. Electronic cigarette liquids present a complex matrix due to the lack of regulation, and therefore standardization, in their manufacturing. A growing trend on internet drug forums involves how to best add drugs of abuse to electronic cigarette liquids for abuse via an electronic cigarette. Due to the lack of published data, development of new analytical methods to accommodate this growing trend was deemed necessary.

GC/MS and LC/MS/MS methods were developed to identify the flavorants of the electronic cigarette liquids as well as identify and quantify nicotine and common drugs of abuse used with these devices.

Seven drugs of abuse were investigated: methamphetamine, heroin, cocaine, fentanyl, and the synthetic cannabinoids JWH-081, JWH-018, and AM-2201. Electronic cigarette liquids from five manufacturers were sampled. From each manufacturer five "flavors" of liquids were chosen. Each liquid "flavor" was tested at the manufacture's reported nicotine concentrations of 0mg/mL, 12mg/mL, and 24mg/mL for a total of 75 electronic cigarette liquid samples.

Liquid-liquid extraction was performed on all samples prior to analysis by GC/MS and LC/MS/MS. Analysis was performed in replicates of five to identify the electronic cigarette liquid components as well as to quantify nicotine and the seven analytes of interest. For any electronic cigarette liquid labeled as containing 0mg/mL of nicotine in which nicotine was identified, the sample was analyzed by GC/MS to quantify the nicotine level. Due to the fact that e-liquids are not regulated, and there is no acceptable concentration of nicotine allowed in a product labeled as containing no nicotine, these concentrations were compared to the naturally occurring levels of nicotine found in certain food products to determine statistical significance.

Identification of the flavorants of the electronic cigarette liquids as well as the quantitation of nicotine and the four commonly abused drugs was accomplished using GC/MS and LC/MS/MS. Nearly all of the flavorants detected have been approved by the Food and Drug Administration for use in food products, but the effects these flavorants have when used via inhalation has not been studied in detail; however, one compound, glutethimide, has not been approved for use in food products and is listed on the Title 21 United States Code Controlled Substance Act as a Schedule II compound.

Samples of e-liquids labeled by the manufacturer as containing 0mg/mL of nicotine may contain detectable and quantifiable levels of nicotine, with concentrations ranging from 31µg/mL to 415µg/mL. Quantitation of drugs of abuse may be affected by matrix components and was found to be dependent on both the specific e-liquid being used with the electronic cigarette as well as the analyte being investigated. For analysis by GC/MS, the e-liquid samples were spiked at a concentration of 150µg/mL with methamphetamine, cocaine, heroin, and fentanyl. Methamphetamine was unable to be quantitated, the calculated concentrations of cocaine ranged from 68.53µg/mL to 177.8µg/mL, the calculated concentrations of heroin ranged from 71.51µg/mL to 197.9µg/mL, and the calculated concentrations of fentanyl ranged from 63.31µg/mL to 211.8µg/mL.

For analysis by LC/MS/MS, the e-liquid samples were spiked at three different concentrations: 40ng/mL, 400ng/mL, and 1,500ng/mL. JWH-081, JWH-018, AM-2201, and heroin were determined to be either inaccurate or imprecise across all three spiked concentrations. Methamphetamine, cocaine, and fentanyl were determined to be the most accurate and precise across all three spiked concentrations, in all e-liquid matrices analyzed by LC/MS/MS.

E-Liquids, GC/MS, LC/MS/MS

K40 Extraction of Selected Barbiturates, Primidone, and Phenytion From Blood Using Supported Liquid Extraction Columns With Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

Gregory A. Priebe, MS, Redwood Toxicology Laboratory, 3650 Westwind Boulevard, Santa Rosa, CA 95403; Brent Dawson, PhD, 353 Hatch Drive, Foster City, CA 94404; Lister M. Macharia, MBA, 3650 Westwind Boulevard, Santa Rosa, CA 95403; and Laureen Marinetti, PhD, Redwood Toxicology Laboratory, Inc, 3650 Westwind Boulevard, Santa Rosa, CA 95403*

After attending this presentation, attendees will understand: (1) the process used for developing a new method for the simultaneous analysis of butalbital, pentobarbital, secobarbital, phenobarbital, primidone, and phenytoin extracted from blood; and, (2) the issues encountered during method validation.

This presentation will impact the forensic science community by developing a method for the extraction and analysis of selected barbiturates, primidone, and phenytoin by GC/MS. Three different extraction schemes were evaluated: Liquid-Liquid Extraction (LLE), Solid Phase Extraction (SPE), and Supported Liquid Extraction (SLE).

This study determined, based on peak area counts, chromatography, and the amount of sample used, that SLE was the best extraction scheme for all analytes of interest. In addition, the SLE columns sped up the extraction process, used less solvents, and used less sample volume when compared to other more traditional extraction schemes (SPE and LLE). The deuterated internal standards used consisted of butalbital-d5, secobarbital-d5, phenobarbital-d5, and phenytoin-d10. Due to either isotopic impurity of the deuterated analog and/or fragmentation characteristics of the parent compound, the secobarbital internal standard was eliminated from this method. The linear dynamic range of each analyte was determined and the calibration curve of each analyte spans this range.

Five hundred microliters of blood containing an internal standard was pretreated with 350µL of 0.1% formic acid and 0.2% ammonium formate in water, then loaded onto the supported liquid extraction columns. A pulse of positive pressure was used to initiate flow, and the sample was allowed to absorb onto the column for five minutes. The sample was then eluted with 2.5mL of dichloromethane and allowed to flow under gravity for five minutes before adding an additional 2.5mL of dichloromethane. The extraction was completed with a short burst of positive pressure. The extraction solvent was evaporated to dryness and the extract was reconstituted in 25µL of ethyl acetate and 25µL of MethElute Trimethylanilinium Hydroxide (TMAH). The reconstituted extract was heated at 70°C for ten minutes, cooled to room temperature, then transferred to auto sampler vials containing inserts. The analysis was performed on a Shimadzu® GC-2010 equipped with an AOC-20i auto injector and fitted with a DB-5MS column (30m x 0.25mm i.d., 0.25µm film thickness). The gas chromatograph was interfaced with a Shimadzu® GC-MS-QP2010S mass selective detector. The injection port and interface temperatures were 250.0°C and 280.0°C, respectively. The oven temperature program was as follows: initial temperature of 95.0°C for one minute and then ramped at 25.0°C per minute to 265.0°C and held for 2.5 minutes.

Method validation followed the Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices for method validation in forensic toxicology. Bias and precision, calibration model, carryover, interference studies, Limit Of Detection (LOD), Limit Of Quantitation (LOQ), dilution integrity, and stability were documented during the validation experiments. Total bias was determined as a mean bias from the target value at three different concentration levels (differing in concentration from calibrator concentrations) over five runs. Total precision was determined as the mean Coefficient of Variation (CV) at three different concentration levels over five runs. The calibration model was determined by the analysis of five replicates each analyzed over five days at six different non-zero calibrator concentrations for each analyte. The data was analyzed using residual plots and the calibration model was determined to be a weighted least squares model. Eight multi analyte interference mixes containing more than 90 compounds at a concentration of 1,000ng/mL were analyzed by the method and no interference was observed. No carryover above the LOD of each analyte was observed at 30,000ng/mL. Additional validation data is summarized in the following table.

Analyte	Avg (%) Bias	Avg %CV	LOD/LLOQ ng/mL	Cal Range ng/mL
Butalbital	0.4	2	200	200-10,000
Pentobarbital	-1.5	3	100	100-5,000
Secobarbital	0.2	8	100	100-2,500
Phenobarbital	-0.2	2	100	200-10,000
Primidone	-0.9	4	500	500-10,000
Phenytoin	-0.8	2	100	200-10,000

Supported Liquid Extraction, Barbiturates, Validation

K41 FAST Analysis of 6-Monoacetyl Morphine (6-MAM) and Acetylcodeine (AC) in Urine of Opiate-Positive Drugs and Driving Cases

Albert A. Elian, MS, Massachussetts State Police Crime Lab, 59 Horsepond Road, Sudbury, MA 01776; and Jeffery Hackett, PhD*, OCME, Forensic Laboratory Division, 850 Bryant Street, N Terrace, San Francisco, CA 94103*

After attending this presentation, attendees will understand a novel yet efficient method for extracting 6-MAM and AC from opiate-positive urine employing commercially available Solid Phase Extraction (SPE) cartridges and analyzing them by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). Metabolism of heroin (diacetylmorphine) is known to proceed via the well-recognized de-acetylation route to 6-MAM, from which morphine and the respective glucuronides (3 and 6 morphine glucuronides) are formed. Heroin use rather than opiate use/misuse may be differentiated by the confirmation of 6-MAM and/or AC in such matrices as urine. With this minimal sample preparation procedure, both 6-MAM and AC can be confirmed in opiate-positive urines by LC/MS/MS for confirmation of heroin use as a novel and efficient alternative to GC/MS.

This presentation will impact the forensic science community by offering analysts operating in forensic facilities information regarding the extraction and analysis of 6-MAM and AC in urine samples obtained in drugs and driving cases using LC/MS/MS. These drugs are used as confirmatory biomarkers for heroin being used by subjects within a short time frame (i.e., within an hour of administration). This information will allow analysts to differentiate opiate use (morphine, codeine) from heroin administration and offer submitting agencies more appropriate interpretation when only urine is the matrix of analysis.

Method: 0.5mL samples of urine (calibrators, controls, and test samples each containing deuterated internal standards) were diluted with 0.5 mL of diluent solution (50% aqueous methanol), vortex mixed, and centrifuged. The supernatant liquid was applied to a mixed mode ion exchange column (3mL, 200mg) and positive pressure was applied (80psi, flowrate 1mL/minute). The eluates were collected in autosampler vials (2mL) for LC/MS/MS. LC was performed in gradient mode employing a 50mm x 2.1mm (3µm) aromatic phase LC column using mobile phase consisting of acetonitrile and 0.1% aqueous formic acid at a flowrate of 0.5mL/minute.

MS/MS was performed in positive multiple reaction (Multiple Reaction Monitoring (MRM)) mode. The following transitions were monitored (quantification transition ions underlined): 6 MAM (328.1 to 165.1 and 211.1), 6-MAM-d_6 (334.1 to 165.1 and 216.1), AC (342.1 to 225.1 and 165.1), AC-d_3 (345.1 to 228.1 and 165.1), respectively In this presentation, representative chromatograms are shown to illustrate the efficiency of the chromatography and analysis of 6-MAM and AC from 20 completed drugs and driving cases.

Results: The limits of detection/quantification for this method were determined to be 0.5ng/mL and 1ng/mL, for 6-MAM and AC, respectively. The method was found to be linear from 1ng/mL to 1,000ng/mL (r^2>0.999). The analyte recoveries were found to be greater than 95% for all of the noted compounds. Inter-day and intra-day variation of the method were found to <8% and <10 %, respectively. Matrix effects were determined to be <6%. Details regarding the concentrations of 6-MAM and AC found in 20 genuine urine cases are presented.

Conclusion: This method demonstrates the efficient use of non-conventional SPE coupled with the use of LC/MS/MS for the analysis of 6-MAM and AC in cases of driving under the influence of drugs. The ability to analyze and confirm these compounds rapidly (i.e., extraction <1 minute per sample and analysis <5 minutes) in drugs and driving cases will clearly assist toxicologists in differentiating between opiates cases involving regular opiates (e.g., morphine, codeine) and those that involve heroin, thus offering the appropriate interpretation to the respective submitting agencies.

6-MAM, Acetlcodeine, LC/MS/MS

K42 Analysis of Opioids in Urine Specimens by Solid Phase Extraction (SPE) and Ultra Performance Liquid Chromatography/Tandem Mass Spectrometry (UPLC/MS/MS)

Melissa A. Johnson, BA, 411 W Bacon Street, Apt 6319A, Richmond, VA 23222; Chinyere M. Williams, BS, 2527 8th Avenue, Apt 211, Oakland, CA 94606; Jeffery Hackett, PhD, OCME, Forensic Laboratory Division, 850 Bryant Street, N Terrace, San Francisco, CA 94103; and Nikolas P. Lemos, PhD, OCME, Forensic Lab Division, Hall of Justice, N Terrace, 850 Bryant Street, San Francisco, CA 94103*

After attending this presentation, attendees will better understand the benefits of using a method combining Enzymatic Hydrolysis (EH), SPE, and UPLC/MS/MS for the routine analysis of opioids in urine.

This presentation will impact the forensic science community by permitting forensic and analytical toxicology laboratories to decrease turnaround time and to increase productivity by introducing a simple, robust, reproducible, and rapid urine opioid procedure based on EH, SPE, and UPLC/MS/MS.

Introduction: In postmortem and human performance forensic toxicology case investigations, body fluids and tissues are routinely submitted to the laboratory for toxicological analysis to help determine if alcohol, drugs, and poisons played any role in the case under investigation. Forensic and analytical toxicology laboratories must, therefore, employ validated methods to screen, confirm, and quantify drugs and their metabolites in biological samples, if present. This study followed method validation guidelines published by the Scientific Working Group for Forensic Toxicology (SWGTOX) to develop and validate a method for the analysis of seven common opioids (morphine (MOR), codeine (COD), 6-acetylmorphine (6-MAM), hydrocodone (HC), hydromorphone (HM), oxycodone (OC), and oxymorphone (OM)) using SPE and UPLC/MS/MS and apply the new methodology to real human urine specimens.

Method: SPE was performed on large particle-size, mixed-mode cartridges (C_8-SCX) to extract the opioids. Urine aliquots (1.0mL) of calibrators, controls, and real case specimens containing internal standard (500ng/mL of a 10µg/mL opiate stock solution of MOR-d6, COD-d6, HC-d6, OC-d6, HM-d6, and OM-d3) were adjusted to pH5 using acetate buffer (1.0M) and hydrolyzed at 65°C for 60 minutes with 50µL of an enzyme derived from Red Abalone. After cooling to room temperature, the pH was adjusted to 6.0 with 3.0mL of aqueous phosphate buffer (0.1M). Samples were vortexed and added to pre-conditioned SPE columns. The columns were washed with 3.0mL each of Deionized (DI) water, acetic acid (1.0M), and methanol and were dried under vacuum for 15 minutes. Analytes were eluted from the SPE cartridges with 3.0mL of solvent mixture (methylene chloride:isopropanol:ammonium hydroxide (78:20:2)). Eluents were evaporated to dryness at 35°C after which the residues were dissolved in mobile phase (100µL) for analysis.

Analyte separation was performed by LC on a pentafluorophenylpropyl column (50mm x 2.1mm, 1.8µm) aided by a guard column. Mobile phases consisted of methanol containing formic acid (0.05%) and DI water containing formic acid/ammonium formate (5mM) (0.05%/0.1%). The flow rate was 0.3mL/minute in a gradient mode. The total run time was nine minutes.

MS/MS was performed in positive Multiple Reaction Monitoring (MRM) mode for morphine (286.2 to 165.1, 201.1), codeine (300.2 to 165.1, 58.2), HM (286.2 to 185.1, 157.1), OM (302.1 to 284.1, 227.1), HC (300.2 to 199.1, 171.1), OC (316.2 to 298.1, 241.1), and 6-MAM (328.2 to 165.1, 211.1). The quantitation ions are underlined.

Results: The method achieved 0.50ng/mL Limit of Detection (LOD) and 2.0ng/mL Limit of Quantitation (LOQ) for all analytes. Linearity was found to be greater than 0.995 in the range from 2.0ng/mL to 1,000ng/mL. Recoveries were determined to be: MOR: 81.25 ± 13.02%; COD: 90.14 ± 9.47%; 6-MAM: 90.18 ± 10.42%; OC: 90.38 ± 11.70%; OM: 88.90 ± 13.43%; HC: 84.64 ± 17.14%; and, HM: 70.06 ± 18.17%.

The intra-day and inter-day variations of the seven opioids were found to be <15% and <12%, respectively. Matrix effects were determined to be: MOR: -34.14 ± 10.37%; COD: -23.869 ± 14.42%; 6-MAM: -24.563 ± 18.12%; OC: -35.813 ± 13.68%; OM: -37.54 ± 16.48%; HC: -24.619 ± 16.71%; and, HM: -37.39 ± 16.55%.

Several closed human performance cases were analyzed and extracted blind. The data were evaluated and compared to the reported results in the database. The results obtained from the new methodology are shown in the provided table.

Specimen #	Drug(s) Found/ (Concentration in ng/mL)
1	MOR (3,972), HM (43.0), HC (1,143), COD (3), OC (1)
2	HM (19), HC (10)
3	MOR (962), HM (16), COD (3,520), HC (22)
4	MOR (21), OM (308), HM (97.0), COD (3), OC (1,102)
5	MOR (66), HM (238), HC (722)
6	MOR (59), HM (308), HC (1,722)

The ability of this new method to detect quantifiable amounts of drugs that were not detected with the current in-house method(s) and are now able to be reported to the database attests to the efficiency of this new procedure.

Conclusion: The newly developed method was used to successfully screen opioids in real forensic toxicology case urine

specimens. The use of SPE and UPLC/MS/MS as part of an extraction procedure that is capable of screening for seven opioids at once can provide valuable data about their presence in urine and cut down turnaround time and analytical costs in high-functioning toxicology laboratories. The method allows laboratories to employ more efficient analyses to simultaneously screen for multiple compounds.

Opioids, Urine, SPE

K43 Comparison of Blood Concentrations for Commonly Encountered Drugs in Postmortem and Human Performance Forensic Toxicology Cases in the City and County of San Francisco

Constantine Konstantakis, BA, San Francisco Medical Examiner's Office, 850 Bryant Street, San Francisco, CA 94103; Tamy Chan, San Francisco Medical Examiner's Office, 850 Bryant Street, San Francisco, CA 94103; Jeffery Hackett, PhD, OCME, Forensic Laboratory Division, 850 Bryant Street, N Terrace, San Francisco, CA 94103; and Nikolas P. Lemos, PhD, OCME, Forensic Lab Division, Hall of Justice, N Terrace, 850 Bryant Street, San Francisco, CA 94103*

After attending this presentation, attendees will be able to better interpret blood drug concentrations in both postmortem as well as human performance forensic toxicology cases.

This presentation will impact the forensic science community by allowing forensic practitioners and others to refer to this study's blood drug concentration data when attempting to evaluate concentrations encountered in their own casework.

In Human Performance (HP) and Postmortem (PM) toxicology case investigations, body fluids and tissues are routinely collected and submitted to the laboratory for toxicological analysis to help determine if alcohol, drugs, and poisons played any role. The interpretation of measured concentrations is often the source of debate and legal challenge, as there exists a limited volume of literature upon which to base opinions and draw inferences. The present study examines and compares blood drug concentrations measured in two groups: (1) living persons (alleged victims or suspects) involved in police investigations (group HP); and, (2) deceased persons who came under the jurisdiction of the San Francisco Office of the Chief Medical Examiner (SFOCME), regardless of case circumstances (group PM). The goal was to assess blood drug concentrations in living and deceased San Franciscans and to better characterize any observed differences between these two groups. Each group theoretically had access to and used similar drug preparations, thereby removing bias based on geolocation and bloods were analyzed by the same American Board of Forensic Toxicologists (ABFT) -accredited laboratory, thus removing analytical-capability bias.

Method: A retrospective examination of the electronic and printed records of the SFOCME from January 2012 to December 2014 was undertaken in order to identify and categorize all subjects of interest. Group 1 subjects gave venous blood (or blood plasma) as part of their case investigation. Peripheral blood specimens were collected at autopsy from Group 2. Blood specimens from both groups were analyzed by the same laboratory using identical procedures for volatiles by headspace gas chromatography and drugs using commercially available screening techniques, including enzyme-linked immunosorbent assay and full-scan gas chromatography/mass spectrometry. Following a positive drug screen result, fresh aliquots of Group 1 bloods and Group 2 peripheral bloods were subjected to confirmations/quantitations utilizing various analytical techniques as required. Commercially available spreadsheet software was used to tabulate and analyze all drug concentrations.

Results: During the three years examined, 2,398 HP and 2,250 PM investigations involving blood drug quantitations were undertaken. A smaller subset of PM cases (n=309) had hospital admission specimens (group AM) associated with them and their blood concentrations were compared to both PM and HP blood concentrations. In groups 1 and 2, ethanol, in %(w/v), was the most commonly encountered substance, ranging from 0.01 to 0.41 (median: 0.16; n=2,073) and from 0.02 to 0.59 (median: 0.09; n=587) in HP and PM cases, respectively. The following table presents blood concentrations in HP and PM cases for the top five most frequently encountered drugs other than ethanol.

Substance (Units)	HP (Blood Range) (median; sample size)	PM (Blood Range) (median; sample size)
THC (ng/mL)	1.0-22.0 (3; n=202)	1.0-81.0 (4; n=261)
Cocaine (mg/L)	0.05-0.30 (0.02; n=30)	0.05-4.0 (0.09; n=114)
Methamphetamine (mg/L)	0.05-2.39 (0.27; n=142)	0.05-9.9 (0.65; n=182)
Methadone (mg/L)	0.05-0.36 (0.24; n=32)	0.05-18 (0.58; n=232)
Morphine (mg/L)	0.05-0.12 (0.06; n=12)	0.05-4.0 (0.17; n=212)

Other drugs commonly encountered included benzodiazepines, hydrocodone, oxycodone, codeine, citalopram, zolpidem, mirtazapine, fentanyl, and trazodone.

Analysis Of Variance (ANOVA) between HP and PM blood concentrations showed statistically significant differences for THC (p=1.96 x 10^{-7}), methamphetamine (p=1.13 x 10^{-6}), and methadone (p=3.58 x 10^{-3}), but cocaine and morphine did not behave in the same manner. When AM blood concentrations of the PM cases were evaluated for variance against HP blood concentrations, methamphetamine was the only drug among the top five most frequently encountered drugs to exhibit statistically significant differences (p=1.49 x 10^{-4}). Finally, statistically evaluating any differences in blood concentrations between AM and PM cases identified methadone as the only substance exhibiting statistical differences (p=8.29 x 10^{-3}).

Conclusion: This study has undertaken a review of blood concentrations in both living and deceased subjects who came under the jurisdiction of the SFOCME. Overall, ethanol, THC, stimulants (cocaine/methamphetamine), opioids, and benzodiazepines were the most commonly encountered substances in both groups. ANOVA identified statistical differences for certain drugs which may be due to sampling differences, postmortem redistribution, and/or postmortem interval. The aforementioned

blood concentration ranges may serve as references to others and allow them to better evaluate drug blood concentration in their case investigations

Postmortem Toxicology, Human Performance Toxicology, Drug Concentrations

K44 Synthetic Cannabinoids in Drivers: Clinical and Psychophysical Indications of Intoxication

Kayla M. Neuman, MS, Wisconsin State Laboratory of Hygiene, 2601 Agriculture Drive, PO Box 7996, Madison, WI 53707-7996*

After attending this presentation, attendees will better understand how synthetic cannabinoids impairment presents in motor vehicle drivers based on Drug Recognition Expert (DRE) evaluations.

This presentation will impact the forensic science community by providing insight into the impairing effects synthetic cannabinoids have on motor vehicle drivers based on Wisconsin Operating While Intoxicated (OWI) casework in addition to how these drugs are being identified in the field by DREs.

Synthetic cannabinoids are increasing in popularity among motor vehicle drivers in Wisconsin; however, there is little known about the clinical and psychophysical effects of these substances. In cooperation with the Wisconsin Bureau of Transportation Safety (BOTS), the Wisconsin State Laboratory of Hygiene sent select whole blood samples to one of two private laboratories for synthetic cannabinoids testing based on information provided by law enforcement agencies. This casework took into account a total of 118 cases from March 2010 to May 2015 in which synthetic cannabinoids use was suspected.

Sixty of the 118 cases (51%) were positive for one or more synthetic cannabinoids and of those, 24 cases (40%) had a DRE evaluation completed with no other drugs detected. These 24 cases, consisting of 20 males (83%) and 4 females (17%), were used to examine the clinical and psychophysical indicators of synthetic cannabinoids impairment based on the information collected during the DRE evaluations. There were a total of 11 different synthetic cannabinoid compounds detected in these 24 specimens, including JWH-018, JWH-019, JWH-022, JWH-122, JWH-210, JWH-250, RCS-4, AM-2201, XLR-11, AB-PINACA, and AB-CHMINACA. The majority of psychophysical and clinical symptoms observed were consistent with the cannabis category of impairment; in keeping with these observations, the primary drug category opined by 83% of DREs was cannabis. Subjects consistently had bloodshot eyes (67%) with droopy eyelids (54%) and a lack of convergence present (75%), as well as impaired balance (54%) and coordination (71%). Horizontal gaze nystagmus was not present in 71% of the subjects and 42% displayed pupil rebound dilation. Other notable impairments were 71% of the subjects exhibited slow speech, 92% swayed from one to six inches during the modified Romberg test, and 75% swayed while balancing during the one-leg stand test. While several psychophysical impairments were exhibited, the majority of the clinical indicators displayed by the subjects were within normal ranges, including pulse rate (54%), blood pressure (63%), body temperature (88%), pupil size (54%), pupil reaction to light (67%), and muscle tone (63%). Initial contact with subjects was primarily due to poor driving and traffic crashes (71%), while 21% were contacted due to an equipment malfunction.

Overall, driver performance on DRE evaluations indicated that synthetic cannabinoids caused significant psychophysical impairment, while most clinical symptoms commonly presented within normal limits. This new data may be used to better enable DRE officers to identify the psychophysical impairment indicative of synthetic cannabinoids use.

Synthetic Cannabinoid, Impairment, DRE

K45 AB-CHMINACA, AB-PINACA, XLR-11, and UR-144 and Driver Behavior in Suspected Impaired Driving Cases in Which a Drug Recognition Expert (DRE) Exam Was Performed

Brittany Thomas, MFS, Washington State Patrol Toxicology Laboratory, 2203 Airport Way, S, Ste 360, Seattle, WA 98134; Brianna Peterson, PhD, 2203 Airport Way, S, Seattle, WA 98134; and Fiona J. Couper, PhD, Washington State Patrol, 2203 Airport Way, S, Ste 360, Seattle, WA 98134*

After attending this presentation, attendees will better understand the driving behaviors of individuals under the influence of the synthetic cannabinoids AB-CHMINACA, AB-PINACA, XLR-11, and UR-144. Additionally, attendees will better understand the various physiological indicators observed by DREs in their exams of individuals under the influence of these synthetic cannabinoids and be able to compare these indicators to those observed with marijuana use.

This presentation will impact the forensic science community by helping law enforcement and toxicologists become familiar with the effects of these synthetic cannabinoids on driving performance, their physiological indicators, how these indicators differ from one compound to another, and how they differ from those observed with marijuana use.

Synthetic cannabinoids are chemically synthesized compounds that are prepared and used to mimic the effects of marijuana.[1] Synthetic cannabinoids are often marketed as legal drugs and are commonly known as "Spice" products. Although attempts have been made to regulate synthetic cannabinoids, modifications to their molecular design provide ways to circumvent scheduling restrictions.[2] In the meantime, people continue to use these drugs and drive.

This presentation reviews case reports for 31 suspected impaired driving cases that were positive for the synthetic cannabinoids AB-CHMINACA, AB-PINACA, XLR-11, and UR-144 and in which DRE exams were performed. All cases were submitted to the Washington State Patrol Toxicology Laboratory from 2012 to 2014, from either Washington State or State of Alaska law enforcement agencies. The population of drivers in this study was predominantly male (95% in AB-CHMINACA and AB-PINACA; 100% in XLR-11 and UR-144), with a mean age of 28 years and 25 years, respectively (range 18-61 years).

Testing for synthetic cannabinoids was performed at either NMS Labs or the American Institute of Toxicology (AIT) laboratory. Horizontal Gaze Nystagmus (HGN) was observed in 50% and 60% of the AB-CHMINACA (*N*=10) and AB-PINACA (*N*=10) cases, respectively. In cases in which XLR-11 (*N*=4), UR-144 (*N*=2), or both (*N*=5) of these compounds were present, HGN was observed in 50%, 0%, and 20% of the cases, respectively. Blood pressure levels were low in the majority of the AB-PINACA, AB-CHMINACA, and UR-144 cases (70%, 60%, and 50%, respectively) and were normal in 100% of the XLR-11 cases. Blood pressure levels were also normal in 80% of the cases in which both XLR-11 and UR-144 were present. Lack of convergence was present in only 30% of the AB-CHMINACA cases and in 0% of the UR-144 cases, but was present in 60%-75% of all the other cases.

Overall, several physiological indicators varied between the four compounds and varied from those typically observed with marijuana use. The majority of these cases had very poor driving skills; subjects were either involved in an accident, found passed out in a vehicle, drove in the wrong direction, or were called in as a suspected impaired driver. Slurred speech, confusion, lack of coordination/dexterity, and lethargy were commonly observed.

Consumption of synthetic cannabinoids can lead to significant impairment, including sedating effects and deficits in fine motor skills which are necessary for safe driving.[3]

Reference(s):

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2. Brents L.K., Prather P.L. (2014) The K2/Spice Phenomenon: emergence, identification, legislation and metabolic characterization of synthetic cannabinoids in herbal incense products. *Drug Metabolism Review*, 46(1): 72-85.
3. Musshoff F., Madea B., Kernbach-Wighton G., Hutter M., Auwärter V. (2014) Driving under the influence of synthetic cannabinoids ("Spice"): a case series. *Int J Legal Med* 128:59-64. DOI 10.1007/s00414-013-0864-1.

Synthetic Cannabinoids, DUID, DRE

K46 XLR-11 and Impaired Driving — Case Reports

Sherri L. Kacinko, PhD, 3701 Welsh Road, Willow Grove, PA 19090; and Barry K. Logan, PhD, NMS Labs/CFSRE, 3701 Welsh Road, Willow Grove, PA 19090*

After attending this presentation, attendees will understand impairments which may be observed in drivers under the influence of the synthetic cannabinoid commonly known as XLR-11.

This presentation will impact the forensic science community by providing new information on the effects of the synthetic cannabinoid XLR-11 on driving, including observations made during the evaluation of drivers by a Drug Recognition Expert (DRE).

The Drug Evaluation and Classification Program was developed to provide a structured series of tests to be performed by DREs to help identify the types of substances, including cannabis, which may be responsible for impairment observed in drivers. While the program is designed to detect the use of marijuana, due to the increasing prevalence of Synthetic Cannabinoids (SCs) in drivers, it must be considered that SCs could be responsible for impairment that is similar to that seen from cannabis use. This presentation includes six cases in which XLR-11, an SC, was identified in blood specimens of drivers who underwent a DRE examination in which only cannabis was identified as being the cause of impairment.

All six drivers indicated they had used SCs. Pulse rate ranged from 50 to 130 beats per minute; two drivers had pulse rates >100 at all three time-points. Horizontal gaze nystagmus, vertical gaze nystagmus, or lack of convergence was not observed in any individual; one had dilated pupils under all light conditions. Two drivers had two clues on the one-leg stand test; the remaining drivers had no clues. Internal clock estimates from the modified Romberg test ranged from 20 to 35 seconds. During the walk-and-turn test, three individuals started too soon, stopped during the exercise, missed heel to toe, stepped off the line, and raised an arm, while two subjects had no observed impairment. Leg, body, and eye tremors were reported for all patients during one or more test. Five of the six individuals had elevated blood pressure and all patients had normal body temperature.

Toxicology tests revealed two individuals to be positive for delta-9 THC and its inactive metabolite, in addition to XLR-11. The delta-9 THC concentrations were low (1.8ng/mL and <1ng/mL). Two drivers tested positive for UR-144 in addition to XLR-11. UR-144 has been identified in herbal incense products and is a predicted metabolite of XLR-11.

In a previous study of 18 drivers, all of whom tested positive for XLR-11 and/or UR-144 and 11 of whom had DRE exams performed, the most common symptoms reported were lack of convergence, slurred speech, and body/eyelid tremors, while blood pressure and pulse rates were noted to be normal.[1] Signs and symptoms indicated on the DRE cover sheets for these six cases were consistent with those typically seen following cannabis use. According to the DRE matrix, elevated blood pressure and pulse rate are consistent with symptoms of cannabis use, which also indicates that after high doses individuals might have dilated pupils. It is important to note that in all six cases, the driver admitted to using a synthetic cannabinoid product, making it difficult to determine if the DRE indicated cannabis use based on the results of the exam or because they were aware these compounds have effects similar to those seen after someone smokes marijuana. These data suggest that XLR-11 may produce impairment that is consistent with impairment generally seen from cannabis and, in cases of low or absent levels of THC, it may be necessary to consider SCs as a potential source of impairment.

Reference(s):

1. Louis A., Peterson B.L., Couper F. XLR-11 and UR-144 in Washington State and state of Alaska driving cases. *Journal of Analytical Toxicology*, 2014 38(8): 563-8.

XLR-11, Driving, DRE

K47 Confirmation of Synthetic Cannabinoids in Driving Under the Influence (DUI) and Sexual Assault (SA) Cases by Liquid Chromatography With Tandem Mass Spectrometry (LC/MS/MS)

Joshua Seither, MS, University of Miami Forensic Toxicology, 1600 NW 10th Avenue, RMSB, R-5, Rm 7025, Miami, FL 33136; and Lisa J. Reidy, PhD, Univ of Miami Forensic Tox Lab, 1600 NW 10th Avenue, RSMB R-5, Rm 7020A, Miami, FL 33136*

After attending this presentation, attendees will understand the application of using LC/MS/MS to identify and confirm synthetic cannabinoids and metabolites in DUI and SA cases. In addition, attendees will learn of drug recognition experts' observations in cases in which synthetic cannabinoid usage was confirmed.

This presentation will impact the forensic science community by providing and demonstrating the applicability of a validated method that can be used to identify a combination of 52 synthetic cannabinoids and metabolites in urine. In addition to the analytical method, drug influence evaluation reports will be presented from cases in which synthetic cannabinoid usage was confirmed.

Synthetic cannabinoid use and popularity has increased over recent years with new synthetic cannabinoid entities entering the drug market frequently. This has created a challenge for law enforcement as it is difficult for many laboratories to update screening and confirmation methods to keep up with the changing drug trends. In an attempt to create a relevant synthetic cannabinoid confirmation method, previous literature and local drug seizure reports were reviewed to generate a list of synthetic cannabinoids and metabolites. From this list, an LC/MS/MS confirmation method was developed and validated.

An enzyme hydrolysis was performed on the urine , followed by liquid/liquid extraction. The extract was then separated by a reverse phase LC gradient method using a Pentafluorophenyl (PFP) column. The run time was 9.5 minutes. A dynamic Multiple Reaction Monitoring (MRM) method was created on an Agilent® 6460 LC/MS/MS in positive electrospray ionization mode. The method targeted a combination of 52 synthetic cannabinoids and metabolites. Method validation was performed following the Scientific Working Group for Toxicology (SWGTOX) and the United Nations Office on Drugs and Crime (UNODC) recommendations. Validation studies included limit of detection, interference, carry over, and matrix enhancement/suppression.

More than one different synthetic cannabinoid was present in each of the positive DUI (seven cases) and SA cases (one case). The synthetic cannabinoids confirmed in these cases were: 5-Fluoro-PB-22 metabolite (one case), AB-CHMINACA (four cases), AB-CHMINACA metabolite (five cases), AM2201 metabolite (one case), JWH-018 metabolite (three cases), JWH-073 metabolite (one case), UR-144 metabolite (five cases), and XLR-11 metabolite (two cases). DRE evaluations were performed on three of these cases.

Typical cannabis impairment indicators include the following: eyelid and body tremors, lack of convergence, and rebound and pupil dilation. Individuals also usually demonstrated an increase in pulse and blood pressure. In order to determine if these indicators are similar to synthetic cannabinoid use, cases with only synthetic cannabinoids or synthetic cannabinoids and cannabis were further analyzed for trends in the DRE evaluations (n=3).

In each of the three cases, the subject had a breath alcohol of 0.00; however, signs of impairment were observed. The subjects shared some similar clinical indicators that aren't considered an indicator of cannabis impairment, such as the presence of horizontal gaze nystagmus, which was observed in all three cases, and the presence of vertical gaze nystagmus was observed in two cases. Two cases displayed lack of convergence. The subjects' blood pressure and pulse were normal for two of the cases and slightly lowered in the third case. One subject had an elevated body temperature. The opinion of the DRE for two of the cases was that of a Central Nervous System (CNS) depressant and CNS stimulant impairment. In the third case, the DRE concluded that impairment was due to a CNS depressant, narcotic analgesic, and cannabis usage. Uncharacteristic cannabis use indicators have been observed in cases in which only synthetic cannabinoid and cannabis use has been confirmed.

Synthetic Cannabinoids, LC/MS/MS, DRE

K48 Indazole-Carboxamide (NACA) Series Synthetic Cannabioids and Driving Impairment

Sherri L. Kacinko, PhD, 3701 Welsh Road, Willow Grove, PA 19090; and Barry K. Logan, PhD, NMS Labs/CFSRE, 3701 Welsh Road, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to describe and discuss the identity and prevalence of NACA compounds in drivers suspected of impaired driving and impairment associated with members of this group of analytes.

This presentation will impact the forensic science community by providing up-to-date information on the current generation of Synthetic Cannabinoids (SC) and their observed impairing effects on drivers.

SCs continue to pose a challenge to law enforcement and toxicologists in terms of establishing which drugs are emerging in drivers being investigated for impaired driving and the nature and severity of observed effects. In addition, and of great interest to the law enforcement community, is the degree of similarity of the impairing effects of the drugs to cannabis, according to the Drug Recognition Expert (DRE) matrix. The composition of SC products is constantly changing. As legislators work to control specific analytes, manufacturers change the drugs in the products in an attempt to evade these legal constrictions. Further, because of their novel nature and their unknown adverse effects, it is impossible to conduct clinical trials of these substances to evaluate their pharmacological properties and potential impairing effects. Consequently, DREs and toxicologists learn about the impairing effects of the drugs through case studies. This presentation describes one of the newest generations of SC designated as the "NACA" series due to their structural similarities, including an indazole-carboxamide group.

Between March 2015 and June 2015, SC testing was completed on 156 blood samples from cases submitted by police agencies. The database of suspected impaired driving cases was queried to identify cases which had tested positive for cases of the NACA drugs. The specific drugs tested by a Scientific Working Group for Toxicology (SWGTOX) -compliant validated method using liquid chromatography/tandem mass spectrometry encompassed: 5F-AB-001, 5F-ADBICA, 5F-ADB-PINACA, 5F-APICA, 5F-APINACA (5F-AKB-48), 5F-MN-18, 5F-PB-22, AB-CHMINACA, AB-FUBINACA, AB-PINACA, ADB-FUBINACA, ADBICA, ADB-PINACA, AM-2201, APICA, APINACA (AKB-48), BB-22, FUB-AKB-48, FUBIMINA, FUB-PB-22, **JWH-018**, **JWH-081**, **JWH-122**, **JWH-210**, MDMB-CHMINACA, MN-18, MN-25, PB-22, THJ-018, THJ-2201, **UR-144**, and **XLR-11**. Analytes in bold were quantified, all others were reported qualitatively. Cases were also tested by immunoassay for other analytes, including opiates, cannabinoids, benzodiazepines, amphetamines, barbiturates, cocaine, methadone, propoxyphene, and PCP. Case 4 in the second table below was also screened for zolpidem, but propoxyphene was not included.

Of the cases described, 74 (47%) were positive for at least one SC, and 23 of the positive cases (31%) contained two or more analytes. The positivity rate for each analyte is presented in the table below.

Analyte	# Positive	% Positive	Conc. (ng/mL)
AB-CHMINACA	35	22.4	
XLR-11	22	14.1	0.21-3.4
AB-FUBINACA	20	12.8	
AB-PINACA	19	12.2	
ADB-FUBINACA	3	1.9	
UR-144	3	1.9	0.34-6.2
ADB-PINACA	1	0.6	
FUB-PB-22	1	0.6	

The following table summarizes three cases which were positive for one or more "NACA" compounds.

Case #	Incident Time	Case Narrative	Blood Collection Time	Toxicology Results
2	22:15	Vehicle sitting in roadway, driver appeared unconscious. Upon awakening, appeared disoriented, slow speech, swaying while standing. Standardized Field Sobriety Tests (SFSTs): Walk and turn – could not keep balance, missed heel to toe, stepped off line; One-Leg Stand – hopped, swayed, raised incorrect foot. No DRE	23:17	AB-PINACA AB-CHMINACA
3*	14:20	Single vehicle crash. Driver interviewed in hospital. Witness accounts of driver crossing center line, leaving roadway, and striking tree. Driver admitted to blacking out due to smoking synthetic marijuana. No SFSTs or DRE.	18:00	AB-FUBINACA

4*	00:49	Passenger vehicle sitting on roadway in drive. Driver appeared to be passed out and upon wakening, appeared confused, sleepy, and uncooperative. SFSTs: Horizontal Gaze Nystagmus (HGN) – 4/6 clues; Walk and turn – could not perform; One-Leg Stand – swayed, raised arms, put foot down. DRE Conclusion: Cannabis and Narcotic Analgesics	02:49	AB-FUBINACA

*Same individual different incidents on different dates

In conclusion, investigators should consider testing for SC compounds in cases in which the history appears consistent with marijuana use, but in which initial testing for THC and its metabolites are negative or cannot explain the observed impairment. The scope of testing needs to be kept up to date with the rapidly changing market for SCs or else the risk of false-negative results for this impairing drug category is high.

Synthetic Cannabinoid, Driver, Impairment

K49 Aligning With the National Safety Council's Recommendations: Redesigning the Enzyme-Linked Immuno-Sorbent Assay (ELISA) Screen Testing Scope and Improving Sensitivity for Driving Under the Influence of Drugs (DUID) Investigation Cases

Ayako Chan-Hosokawa, MS, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090*

After attending this presentation, attendees will better understand how to evaluate prevalency data, the changes in drug trends, and observed blood drug concentrations in impaired driving investigation cases to optimize the routine immunoassay screen ELISA.

This presentation will impact the forensic science community by providing information regarding constantly changing drug trends and the drug screen positivity rates over the years in DUID investigations and by demonstrating the need to update the ELISA drug screen.

With an increasing awareness of the high prevalence of drug use in drivers, both the Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID) and the National Safety Council (NSC) published a set of standardized guidelines for toxicological investigation of drug and impaired driving and motor vehicle fatalities. Based on the 2012 recommendations by the NSC, the scope and sensitivity of DUID screening and confirmation procedures at NMS Labs were evaluated and optimized.

The scope of screen and confirmation tests as well as cut-off concentrations for blood, urine, and oral fluid in NMS Lab's DUID panel were assessed and determined to be mostly in agreement with the new recommended guidelines. To align with the NSC's recommendations, a few improvements were proposed: (1) removal of propoxyphene from the ELISA screen; (2) inclusion of zolpidem and carisoprodol to the ELISA screen; (3) improvement of the ELISA screen sensitivity for low-dose benzodiazepines such as lorazepam and clonazepam; and, (4) improvement of confirmation cut-offs for opiates (i.e., 6-monoacetylmorphine, oxymorphone, and hydromorphone). Each of the proposed improvement projects was further supported by the prevalence study, as well as observed blood drug concentrations and/or therapeutic drug concentrations.

The use of propoxyphene has significantly decreased since 2010, when the compound was banned by the Food and Drug Administration (FDA). The positivity rate of 0.11% (44 out of 39,260 cases) in the past five years (since January 2010) was the lowest among the compounds included in the ELISA screen. Thus, propoxyphene was removed from the ELISA screen scope and moved to an expanded therapeutic drug screening scope using a mass spectrometry technique.

In addition, the popularity of the compounds zolpidem, carisoprodol and its metabolite meprobamate in DUID cases was determined by calculating positivity rates from the expanded therapeutic drug screening. Of 167 compounds included in the scope of the analysis, zolpidem, meprobamate, and carisoprodol were the fifth (5.78%), seventh (5.41%), and eighth (5.22%) most frequently reported compounds during this study period. The list of the top 15 drugs found in 2013 DUID cases provided by the Pennsylvania Traffic Safety Resource Prosecutor (TSRP) also included carisoprodol and zolpidem. Based on these prevalence studies, both carisoprodol and zolpidem were upgraded to the ELISA screen scope.

Traditionally, benzodiazepine ELISA plates are designed using oxazepam as a reference and relying on cross-reactivity of other benzodiazepines to trigger a confirmation. Due to known poor cross-reactivity of clonazepam and lorazepam, a cut-off concentration of 100ng/mL of oxazepam was not sensitive enough to detect blood drug concentrations within the therapeutic ranges for these compounds. Based on the data analysis using both screening methodologies (ELISA and **Liquid Chromatography/Time-of-Flight**/Mass Spectrometry (LC/TOF/MS)), it was also determined that approximately 3% of benzodiazepines positive cases were initially not detected. A cut-off concentration of 20ng/mL was achieved by utilizing a calibration standard in 5-folds dilution. As a result, the ELISA benzodiazepine positivity rate increased from 13.9% to 23.5% in the first month, making benzodiazepines the second most-prevalent compound after cannabinoids in DUID investigation cases.

Lastly, opiates confirmatory analysis was redeveloped on Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) to accommodate the low cut-off concentrations that are consistent with the therapeutic ranges for oxymorphone and hydromorphone as well as low 6-monoacetylmorphine blood concentrations detected due to rapid metabolism. The cut-offs were improved from 10ng/mL to 5.0ng/mL for codeine, dihydrocodeine/hydrocodol, hydrocodone, morphine, and oxycodone and to 1.0ng/mL for 6-monoacetylmorphine, oxymorphone, and hydromorphone. As expected, the significant increase in positivity rates was observed for many compounds: oxymorphone (1.00% to 13.8%), morphine (38.4% to 48.9%), and 6-monoacetylmorphine (2.10% to 12.2%).

Drug-impaired driving has been a noted problem for many years with guidelines that were developed back in the 1980s; however, it was recognized that this is not a static problem and therefore toxicology laboratories must evaluate and adjust their testing scopes to maintain relevancy to modern times.

DUID Drug Screen, Standardized Guideline, ELISA

K50 Statistical Assessment of Toxicology Cases Submitted to the Las Vegas Metropolitan Police Department (LVMPD) From 2000 Through 2014

Michael P. Stypa, MS, 5605 W Badura Avenue, Ste 120B, Las Vegas, NV 89118; Denise K. Heineman, BS, Las Vegas Metropolitan Police Department, 5605 W Badura Avenue, Ste 120B, Las Vegas, NV 89118; Darby A. Lanz, MSFS, 5605 W Badura Avenue, Ste 120B, Las Vegas, NV 89118; and Jennifer O. Rattanaprasit, MS, Las Vegas Metropolitan Police Department, Forensic Lab, 5605 W Badura Avenue, #120B, Las Vegas, NV 89118*

After attending this presentation, attendees will better understand the statistical trends in Las Vegas DUI alcohol and drug cases over a 14-year period.

This presentation will impact the forensic science community by serving as a statistical reference for the occurrence of alcohol and drugs in DUI cases submitted to the LVMPD over an extended time period. Moreover, the dynamics of a large tourist population and its relationship to trends in DUI casework may be of interest to cities with similar demographics.

Las Vegas is an international tourist destination known worldwide for its dining and entertainment. The LVMPD serves the law enforcement needs of a jurisdictional population of 1.5 million residents and 40 million annual visitors. The majority of the toxicology requests submitted to the LVMPD are categorized as human performance Driving Under the Influence (DUI) cases. Toxicology statistics were evaluated for cases submitted to the laboratory from 2000 through 2014. The specific areas of interest included in this study were breath alcohol, blood alcohol, and blood drug analyses.

DUI alcohol casework constituted the majority of all toxicology requests at the LVMPD. An average of 2,300 breath alcohol tests were conducted annually between 2000 and 2008. The number of breath alcohol tests increased to 3,205 in 2009, peaked at 3,746 in 2010, and has held steady since that time. Blood alcohol casework numbered 2,630 at the beginning of this study and gradually increased to a maximum of 7,824 in 2009. After 2009, the number of blood alcohol cases progressively decreased to a total of 3,488 in 2014. Statistics for blood drug casework follow a similar pattern as the blood alcohol data. There were 782 blood drug cases in 2000. The number of blood drug cases increased to a maximum of 3,156 in 2011 before declining to 1,753 in 2014.

The gradual increase in toxicology requests from 2000 through 2008 mirrored a growing population and the hiring of additional police officers to meet population demands. The notable increase in the number of breath alcohol tests beginning in 2009 corresponded to a strategy by the LVMPD to encourage more economical breath alcohol analysis during the Great Recession. A significant decrease in blood alcohol and blood drug requests occurred in 2013. The LVMPD police force declined in 2013 when hiring did not keep up with attrition. Another contributing factor to the drop in blood casework may be a United States Supreme Court decision (*Missouri v. McNeely*, 2013) requiring a search warrant prior to drawing blood from persons suspected of DUI.[1]

Alcohol concentration data were consistent over the course of this study. A detailed look at DUI alcohol statistics revealed that 10% of cases involved drivers with alcohol concentrations less than the 0.08% illegal limit. A large percentage of subjects had alcohol concentrations between 0.08% and 0.16% (50% of breath alcohol cases, 33% of blood alcohol cases). A remarkably high percentage of individuals cited for DUI had elevated alcohol levels of 0.16% and higher (40% of breath alcohol cases, 55% of blood alcohol cases). The large percentage of drivers with elevated alcohol concentrations is alarming because these individuals represent a very high risk to public safety.

The majority (>40%) of all DUI drug cases submitted to the LVMPD were positive for a single drug/drug class. There has been an increase in the percentage of poly drug cases over the years and these types of cases currently represent 30% of the total. The percentage of cases where no drugs were detected has declined and is currently tracking at 20%. Cannabis is the most commonly occurring drug found in Las Vegas DUI cases. Interestingly, the prevalence of cannabis has held steady throughout this study and accounts for approximately 38% of all drugs reported. Benzodiazepines represent the second most prominently occurring drug type.

Reference(s):

1. *Missouri v. McNeely* 569 U.S. ___ (2013).

Las Vegas, Forensic Toxicology, DUI

K51 Zolpidem Concentrations Found in 644 Blood Samples Submitted for Driving Under the Influence of Drugs (DUID) Analysis

Lee M. Blum, PhD, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090; and Laura M. Labay, PhD, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090*

After attending this presentation, attendees will be better informed regarding zolpidem and the blood concentrations measured in a large population of drivers investigated for DUID.

This presentation will impact the forensic science community by providing additional information that may be of benefit to toxicologists, pathologists, and investigators when evaluating similar types of cases.

Zolpidem (Ambien®) is a sleep-aid classified as a non-benzodiazepine hypnotic of the imidazopyridine class that is prescribed for the short-term treatment of insomnia. Because of its sedative-hypnotic properties, the drug has often been implicated with impaired driving, drug-facilitated crimes, and lethal outcomes. Some common effects associated with zolpidem use include dizziness, headache, and nausea, while rare effects include somnambulism and anterograde amnesia. Cases have been reported in which people, after taking zolpidem, performed complex tasks such as eating, shopping, or driving, but then had little or no memory about the events after awakening. Due to the frequency of zolpidem use and its potential adverse effects profile, it is relevant to evaluate blood drug concentrations in drivers.

Over the course of an approximate 4-year period, 644 blood samples have been tested for zolpidem in specimens submitted for toxicology testing as part of the DUID investigation process. The test is briefly described as follows: blood samples underwent a four-fold dilution prior to mixing with a deuterated internal standard (zolpidem-d6) and 1% phosphoric acid. Zolpidem was then extracted through a solid phase extraction procedure. Eluents were evaporated to dryness, subsequently reconstituted with 50% Deionized (DI) water/50% Mobile Phase, and supernatants were centrifuged prior to transfer to autosampler vials. Analysis was achieved using reverse phase High-Performance Liquid Chromatography (HPLC) separation with positive-ion Electrospray Tandem Mass Spectrometry (ES/MS/MS) for detection and quantification. The ions monitored for zolpidem were 308.0m/z to 235.0m/z and 263.0 m/z, respectively. The lower limit of quantification was 4.0ng/mL with an analytical measurement range of 4.0ng/mL-800ng/mL.

A review of the data set revealed that these samples were positive across a wide concentration range (i.e., from less than 4.0ng/mL to 2,000ng/mL). The mean ± Standard Deviation (SD) and median from 636 positive results were 255ng/mL ± 286ng/mL and 150ng/mL, respectively. As a point of reference, plasma concentrations following single oral 5mg and 10mg doses occur at approximately 1.6hrs following ingestion and range from 29ng/mL to 113ng/mL for the 5mg dose and 58ng/mL to 272ng/mL for the 10mg dose. Most results are, therefore, consistent with therapeutic concentrations. The average age by gender was 44.7yrs (range: 21yrs-71yrs; median: 45yrs) for females (*n*=170), and 46.1yrs (range: 19yrs-87yrs; median: 46yrs) for males (*n*=201). In 2013, the Food and Drug Administration informed manufacturers that the recommended dose of zolpidem for women should be lowered from 10mg to 5mg for immediate-release products and from 12.5mg to 6.25mg for extended-release products. Differentiating this data by gender showed an average concentration in females (*n*=176) of 271ng/mL ± 300ng/mL (range, 5.6 g/mL-1,400ng/mL) with a median value of 155ng/mL. In males (*n*=201), the average concentration was 222ng/mL ± 245ng/mL (range, 4ng/mL-1,700ng/mL) with a median value of 140ng/mL. Of the 636 positive cases, 79 were positive for only zolpidem. In this subset of cases, the average concentration was 314ng/mL ± 321ng/mL (range, 5.9ng/mL-1,700ng/mL) with a median value of 200ng/mL. Other drugs identified in the entire population included amphetamines (e.g., methamphetamine), benzodiazepines (e.g., diazepam, alprazolam, clonazepam, and lorazepam), and opiates (e.g., oxycodone, hydrocodone, and fentanyl), among others.

This data demonstrates that zolpidem is associated with driving impairment across a wide concentration range. Because zolpidem is a sleep aid, concentrations consistent with therapeutic concentrations would not be considered compatible with the safe operation of a motor vehicle. Concentrations that are well above concentrations associated with prescribed dosages imply tolerance to the medication. Per research, these data represent the largest population of zolpidem positive specimens that has been evaluated in regard to the impaired operation of a motor vehicle.

Zolpidem, DUID, Impaired Driving

K52 Methamphetamine and Amphetamine in Suspected Driving Under the Influence (DUI) Cases in the City and County of San Francisco: A Six-Year Review

Eric A. Ingle, BA, 1965 Pleasant Hill Road, Pleasant Hill, CA 94523; Mariya Mayevskaya, BA, Office of the Chief Medical Examiner, 850 Bryant Street, Hall of Justice, N Terrace, San Francisco, CA 94103; Justin A. Volk, OCME, 850 Bryant Street, North Terrace, San Francisco, CA 94103; Jonas E. Knight, MS, Hall of Justice-North Terrace, 850 Bryant Street, San Francisco, CA 94103; Pavlos Karamanidis, BS, 407 Sanchez Street, Apt 3120, San Francisco, CA 94114; Glenda M. Easterling, BS, OCME, 850 Bryant Street, San Francisco, CA 94103; Chinyere M. Williams, BS, 2527 8th Avenue, Apt 211, Oakland, CA 94606; Jeffery Hackett, PhD, OCME, Forensic Laboratory Division, 850 Bryant Street, N Terrace, San Francisco, CA 94103; and Nikolas P. Lemos, PhD, OCME, Forensic Lab Division, Hall of Justice, N Terrace, 850 Bryant Street, San Francisco, CA 94103*

After attending this presentation, attendees will possess the necessary knowledge to assess methamphetamine/amphetamine blood concentrations in DUI cases based on a six-year review of such cases in San Francisco.

This presentation will impact the forensic science community by demonstrating the need for and usefulness of comprehensive drug testing in DUI cases irrespective of the driver's alcohol concentration and will provide reference concentrations for others to use in their own case work.

In the City and County of San Francisco, the American Board of Forensic Toxicologists (ABFT) -accredited Forensic Laboratory Division (FLD) of the Office of the Chief Medical Examiner (OCME) performs all forensic toxicology testing, including postmortem as well as human performance forensic toxicology cases. A retrospective review of all suspected DUI cases submitted to the FLD between April 1, 2009, and April 30, 2015, was undertaken in order to better understand and characterize the incidence of methamphetamine/amphetamine (mAmp/Amp) in DUI cases in San Francisco.

Prior to August 1, 2014, DUI specimens were only screened for drugs by immunoassay and/or gas chromatography/mass spectrometry if drug testing was specifically requested by the police or if the ethanol was ≤0.12%(w/v). Since August 1, 2014, all DUI specimens have been screened for drugs, regardless of the driver's blood alcohol concentration.

The yearly distribution of mAmp/Amp DUI cases in San Francisco are shown below:

Period	No. of mAmp/Amp Cases
Apr. 1 to Dec. 31, 2009	7
Jan. 1 to Dec. 31, 2010	17
Jan. 1 to Dec. 31, 2011	37
Jan. 1 to Dec. 31, 2012	37
Jan. 1 to Dec. 31, 2013	36
Jan. 1 to Dec. 31, 2014	40
Jan. 1 to Apr. 30, 2015	21

It is noteworthy that in 2014 and the first part of 2015, the number of mAmp/Amp DUI cases have increased as the FLD's testing protocol was adjusted in compliance with the "Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities."[1]

Examination of digital and physical records revealed that in the six-year period of interest, the FLD has performed analyses in 5,023 DUI cases (494 felonies; 4,529 misdemeanors). Of these, 179 cases (3.6%) had mAmp/Amp in blood and of those, there were 66 cases in which mAmp/Amp were the only drugs found in blood.

Whites, Hispanics, and Blacks comprised the three most common race groups among the 179 drivers with mAmp/Amp in their blood and among the 66 drivers with only mAmp/Amp in their blood.

One hundred thirty-seven of the 179 drivers with mAmp/Amp were male (76.5%). The mean and median age of the 179 drivers with blood mAmp/Amp was 37 years and 35 years, respectively. In the 179 cases with blood mAmp/Amp, the mean and median mAmp concentrations were 355ng/mL and 250ng/mL, respectively, while the mean and median Amp concentrations were 54ng/mL and 50ng/mL, respectively.

Fifty-two of the 66 drivers with only mAmp/Amp in their blood were male (78.8%). The mean and median age of these 66 cases were 39 years and 40 years, respectively. In the 66 cases with only mAmp/Amp in their blood, the mean and median mAmp concentrations were 454ng/mL and 320ng/mL, respectively, while the Amp concentrations were 56ng/mL and 50ng/mL, respectively.

In the 113 cases with other substances detected, the most common ones were cannabinoids (n=59; 52.2%), ethanol (n=25; 22.1%), and benzodiazepines (n=24; 21.2%). Other compounds detected included GHB, MDMA, PCP, cocaine, methadone, carisoprodol/meprobamate, morphine/codeine/6-MAM, hydrocodone, oxycodone, zolpidem, and mirtazapine.

Analysis of variance revealed statically significant differences (p<0.05) when the mAmp/Amp-only drivers' sex was examined for age: males' mean age was 39.97 years, but females' mean age was 33.92 years. Differences were also noted in the

time of day the blood collections took place. Most specimen collections from female drivers occurred from 9:00 p.m. to 2:59 a.m., peaking between 1:00 a.m. and 2:59 a.m. (11 of 40 cases, 27.5%), but most collections for male drivers occurred between 7:00 p.m. and 4:59 a.m., peaking between 3:00 a.m. and 4:59 a.m. (21 of 137 cases, 15.3%).

This retrospective review of mAmp/Amp DUI cases in San Francisco clearly demonstrates that methamphetamine DUIs remain an issue in our driver population and comprehensive drug screening beyond alcohol in these cases is justified in the interest of public safety, despite the perceived short-term financial burden and time delays associated with drug testing.

Reference(s):

1. Logan B.K., Lowrie K.J., Turri J.L., Yeakel J.K., Limoges J.F., Miles A.K., et al, Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities. *Journal of Analytical Toxicology* 2013;37:552–558.

Methamphetamine & Amphetamine, DUI, San Francisco

K53 Blood Cannabinoid Pharmacokinetics in Frequent Cannabis Smokers After Controlled Smoked, Vaporized, and Oral Cannabis Administration: Markers of Recent Cannabis Intake

Matthew N. Newmeyer, BS, National Institute on Drug Abuse, 251 Bayview Boulevard, Ste 200, Rm 05A721, Baltimore, MD 21224; Karl B. Scheidweiler, PhD, NIDA-IRP, NIH, 251 Bayview Boulevard, Ste 200, Rm 05A729, Baltimore, MD 21224; Allan J. Barnes, BS, 5500 Nathan Shock Drive, Rm 373, Baltimore, Maryland 21224; Agnes O. Coffay, MD, Office of the Clinical Director, National Institute on Drug Abuse, NIH, 251 Bayview Boulevard, Baltimore, MD 21224; Osama A. Abulseoud, MD, Chemistry and Drug Metabolism, National Institute on Drug Abuse, NIH, 251 Bayview Boulevard, Baltimore, MD 21224; and Marilyn A. Huestis, PhD, Chemistry & Drug Metabolism, Intramural Research, NIDA, NIH, 251 Bayview Boulevard, Rm 05A721, Baltimore, MD 21224*

After attending this presentation, attendees will be able to describe the blood pharmacokinetics of Δ^9-Tetrahydrocannabinol (THC), its metabolites, and minor cannabinoids after controlled smoked, vaporized, and oral administration in frequent cannabis smokers.

This presentation will impact the forensic science community by aiding in the interpretation of blood cannabinoid results after three different administration routes within the same participants.

Cannabis is the most commonly reported illicit drug in motor vehicle crashes and fatalities. THC is rapidly distributed into highly perfused organs and later into adipose tissue. With frequent cannabis intake, a large body burden of THC is achieved that slowly re-enters the bloodstream, such that positive blood cannabinoid results are observed hours after the window of acute impairment, complicating cannabinoid result interpretation. There is increasing interest in whether unique cannabinoid markers (cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), Δ9-tetrahydrocannabivarin (THCV), 11-nor-9-carboxy-THCV (THCVCOOH), and THC-glucuronide) can identify recent cannabis intake after it is smoked, vaporized, and/or ingested. Direct comparison of cannabinoid pharmacokinetics in the same population after different administration routes has not been investigated.

Seven frequent (≥5x/week) cannabis smokers participated in this National Institute on Drug Abuse Institutional Review Board, Food and Drug Administration (FDA), and Drug Enforcement Administration (DEA) -approved study; all provided written informed consent. Participants entered the secure research unit approximately 19h prior to dosing. The study was conducted with a double-blind, crossover, and placebo-controlled design consisting of four sessions that were randomized, including a double-placebo session. Participants consumed a placebo or active oral (baked in a brownie) cannabis dose (6.9% THC), followed by placebo or active smoked or vaporized cannabis. Only one administration route had active THC in each session. Smoking, inhaling, and eating occurred *ad libitum* for 10min. Blood was collected prior to, during, and up to 72h after dosing. THC, 11-hydroxy-THC (11-OH-THC), 11-nor-9-carboxy-THC (THCCOOH), CBD, CBN, CBG, THCV, THCVCOOH, THC-glucuronide, and THCCOOH-glucuronide concentrations were quantified by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). Wilcoxon signed-rank tests were performed to compare median pharmacokinetic parameters between administration routes (two-tailed $p<0.05$ significance threshold).

A total of 189 blood specimens were collected for each active administration route. THC was quantifiable in all specimens collected after smoking and oral dosing, and 92.1% after vaporization (THC only up to 5h post-dose in one participant). 11-OH-THC was measureable in 64.6%-70.9% of specimens and THCCOOH in all specimens. Median THC, 11-OH-THC, and THCCOOH times (t_{max}) of maximum observed concentrations (C_{max}) were 2.5h after oral dosing and occurred significantly later than after smoking (0.10h, 0.20h, and 0.25h, respectively) or vaporization (0.10h, 0.17h, and 0.25h, respectively). Median THC C_{max} was significantly greater after smoking (117µg/L) and vaporization (98.0µg/L) than oral dosing (15.6µg/L). Median THCCOOH C_{max} after oral dosing (75.2µg/L) was significantly greater than after vaporization (29.5µg/L). THCCOOH-glucuronide was quantifiable in all specimens after smoking and oral dosing, and 91.5% after vaporization; THCCOOH was detected longer than THCCOOH-glucuronide after vaporization due to differences in limits of quantification. CBD, CBN, CBG, and THCV were only observed after smoking and vaporization (7.4%-30.2%); median last detection times (t_{last}) were 0.17h-0.5h, except for 1.5h after smoking for CBN. THCVCOOH was measured in 10.6%-21.7% of specimens after all doses, but only in all participants after oral doses. THCVCOOH was first quantified a median of 1.5h after oral dosing, but detectable for as long as 26h. THC-glucuronide was quantifiable in 1.6% of specimens after smoking (t_{last} 0.5h) and 3.2% after oral (t_{last} 2.5h) cannabis.

Cannabinoid pharmacokinetics after smoking and vaporization were similar, but significantly different from oral dosing. CBN, CBG, and THCV were good markers of recent cannabis smoking and vaporization when identified, but were not measureable in all participants. THC-glucuronide was present in fewer specimens than other markers, but detected after oral dosing when others were not. Presence of CBN, CBG, THCV, or THC-glucuronide identified cannabis intake within 2.5h after any route of administration, but absence of any of these markers did not preclude recent cannabis use. These data improve identification of recent cannabis intake and improve interpretation of blood cannabinoid results.

Supported by the National Institute on Drug Abuse, IRP, National Institutes of Health

Cannabis, Administration Routes, Pharmacokinetics

K54 Neurocognitive Performance in Occasional and Frequent Smokers Following Controlled Smoked, Vaporized, and Oral Cannabis Administration

Madeleine J. Swortwood, PhD, National Institute on Drug Abuse, 251 Bayview Boulevard, BRC 05A721, Baltimore, MD 21224; Matthew N. Newmeyer, BS, 304 Drew Street, Baltimore, MD 21224; Agnes O. Coffay, MD, Office of the Clinical Director, National Institute on Drug Abuse, NIH, 251 Bayview Boulevard, Baltimore, MD 21224; Osamax A. Abulseoud, MD, Chemistry and Drug Metabolism, National Institute on Drug Abuse, NIH, 251 Bayview Boulevard, Baltimore, MD 21224; and Marilyn A. Huestis, PhD, Chemistry & Drug Metabolism, Intramural Research, NIDA, NIH, 251 Bayview Boulevard, Rm 05A721, Baltimore, MD 21224*

After attending this presentation, attendees will be able to describe cannabis' effect on neurocognitive performance after smoked, vaporized, and oral cannabis administration to occasional and frequent cannabis smokers.

This presentation will impact the forensic science community by aiding in the interpretation of neurocognitive performance after three different administration routes in two groups of cannabis smokers.

The objective of this study was to evaluate cannabis' neurocognitive effects in occasional and frequent smokers after three different administration routes. Cannabis remains the most commonly used illicit substance in the world and was the most prevalent illicit drug detected in 12.6% of nighttime drivers in the 2013-2014 National Roadside Survey. Cannabis smoking is associated with poor driving performance and approximately doubles the risk of involvement in a motor vehicle accident. Although smoking is the most common cannabis administration route, oral consumption and cannabis vaporization are also popular; however, data on the impact of delivery mode and intake frequency on cannabis' pharmacodynamic effects remain limited.

Eight frequent (≥5x/week) and eight occasional (≥2x/month but <3x/week) adult cannabis smokers were recruited to participate in this National Institute on Drug Abuse Institutional Review Board, Food and Drug Administration (FDA), and Drug Enforcement Administration (DEA) -approved study; all participants provided written informed consent. Participants entered the secure research unit approximately 19h prior to dosing to preclude acute intoxication. Sessions followed a double-blind, double-dummy, randomized, crossover, placebo-controlled design. Over the course of four dosing sessions, participants consumed a placebo or active oral (baked in a brownie) cannabis dose (6.9% THC), followed by either placebo or active smoked or vaporized cannabis. Only one route of administration had active THC in each session. Smoking, inhaling, and eating were each performed *ad libitum* for 10min. Participants were trained before study sessions to achieve stable task performance. Neurocognitive tasks were performed at -1.5h (baseline), 0.5h, and 2.5h relative to the start of dosing. The Stop Signal Task (SST) measures motor impulsivity, or the inability to inhibit a pre-cued response. The Tower of London (TOL) task is a decision-making task measuring executive function and planning. Friedman's Analysis of Variance (ANOVA) and Wilcoxon tests were utilized to examine within-group effects while Mann-Whitney tests were executed to examine between-group effects. Statistical significance was attributed at $p<0.05$, with trends attributed at $p<0.1$.

In the SST, there was a significant group effect between frequent and occasional smokers for all administration routes, indicating poorer performance in frequent smokers. Frequent smokers demonstrated significantly lower total accuracy ($p=0.0180$) than occasional smokers 2.5h after vaporization with a trend at 0.5h ($p=0.0512$). A trend also was observed ($p=0.0512$) for frequent smokers' lower total accuracy compared to occasional smokers 2.5h after smoking. Trends in longer reaction times in no-stop trials at 0.5h ($p=0.0939$) and 2.5h ($p=0.0721$) after oral administration in occasional smokers were observed. For frequent smokers, the SST was sensitive to cannabis' impairing effects as indicated by lower accuracies after smoking and vaporization. For occasional smokers, the SST was sensitive to oral cannabis' impairing effects as indicated by longer reaction times in no-stop trials.

For TOL, there were no significant effects in frequent cannabis smokers on total score or task completion time after smoked or oral cannabis administration. Frequent smokers had no significant change in total score, but completed the task significantly faster ($p=0.0371$) 0.5h after vaporization compared to baseline with a trend observed between baseline and 2.5h ($p=0.0645$). A significant ($p=0.0375$) overall time effect was observed for occasional smokers' decrease in completion time after smoking. Other factors, such as consumed *ad libitum* dose, tolerance, and baseline neurocognitive function may play a role in task performance. These data have implications for driving under the influence of cannabis, as reaction time and motor impulsivity impact driving performance.

Supported by the National Institutes of Health, IRP, National Institute on Drug Abuse.

Cannabis, Administration Route, Neurocognitive Impairment

K55 Comparison of the Randox® Evidence Drugs of Abuse Custom Array VIII Biochip With Accurate Mass Screening III: Meprobamate (MPB), Methadone (MTD), Tramadol (TRM), and Zolpidem (ZPD)

Daniel S. Isenschmid, PhD, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090; Denice M. Teem, BS, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090; Samantha Beauchamp, BA, Michigan State Police Forensic Laboratory, 7320 N Canal Road, Lansing, MI 48913; Geoffrey French, BS, Michigan State Police Forensic Laboratory, 7320 N Canal Road, Lansing, MI 48913; Lindsay Rohrbacher, BS, Michigan State Police Forensic Laboratory, 7320 N Canal Road, Lansing, MI 48913; Mark Vandervest, BA, Michigan State Police Forensic Laboratory, 7320 N Canal Road, Lansing, MI 48913; and Jennifer S. Wilson, BS, Michigan State Police Forensic Laboratory, 7320 N Canal Road, Lansing, MI 48913*

After attending this presentation, attendees will better understand the comparison of the results obtained between the custom biochip assays for MPB, MTD, TRM, and ZPD with Liquid Chromatography/Time-Of-Flight (LC/TOF) accurate mass screening and Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) or Gas Chromatography/Mass Spectrometry (GC/MS) confirmation.

This presentation will impact the forensic science community by allowing attendees to assess the usefulness of the several assays incorporated in the Randox® Evidence Drugs of Abuse Custom Array VIII Biochip for drug screening in Driving Under the Influence of Drugs (DUID) cases.

Introduction: Blood specimens collected in suspected DUID cases in the State of Michigan are routinely screened for drugs by the Michigan State Police using a Randox® Evidence Analyser and a Drugs of Abuse Custom Array VIII Biochip employing chemiluminescent immunoassay technology. The custom chip is embedded with 14 different antibodies to desired target analytes in discrete testing regions. As part of a workload reduction project, specimens that screened positive for one or more analytes on the biochip were sent to NMS Labs for analysis by LC accurate mass screening and confirmation of presumptive positive findings.

Methods: Blood specimens were analyzed with cutoff concentrations as noted in Table 1 using the Randox® Biochip as the initial screen, LC/TOF accurate mass screening as a rescreen, and confirmations by either LC/MS/MS or GC/MS. Only cases which tested positive above the LC/TOF decision point were confirmed.

Table 1: Biochip cut-off concentrations, LC/TOF Decision Points and LC/MS/MS, and GC/MS Reporting Limits (ng/mL)

	Target Analytes (ng/mL)						
Method	**Meprobamate**	**Carisoprodol**	**Methadone**	**EDDP**	**Tramadol**	**O-DMT**	**Zolpidem**
Biochip	25	*	10	*	5	*	5
LC/TOF	1000	200	50	50	20	25	10
LC/MS/MS	-	-	-	-	20	20	4
GC/MS	1000	200	50	50	-	-	-

*The manufacturer reported cross-reactivities of 88% for carisoprodol (MPB assay), <0.01% for EDDP and EDMP (MTD assay), 32.8%, 11.9%, 2.7% for the (+/-)N,O didesmethyl, O-desmethyl, (+/-)N-desmethyl tramadol metabolites (TRM assay), and 31% for phenyl-4carboxy zolpidem (ZPD assay).

Results: A total of 1,858 blood specimens were tested. Table 2 summarizes the data obtained by the biochip assays and the LC/TOF screen. Although the LC/TOF decision point for the MPB assay had a much higher decision point than the Biochip cutoff, false negatives were minimized due to the cross-reactivity of carisoprodol, which was present in 94% of the positive cases. For all analytes, most cases for which the Biochip was positive and the LC/TOF was "negative" had an LC/TOF response for the drug, but below the decision point; however, since LC/TOF was considered the screening test for testing purposes at NMS Labs, cases with analytes below the LC/TOF decision point were not confirmed and were not considered as true positives in the calculations. For reference, the values in Table 2 in parenthesis are "true" false positive cases (positive Biochip, no LC/TOF response). All positive results by LC/TOF were confirmed by LC/MS/MS or GC/MS.

Table 2: Results: Biochip and LC/TOF screen (parenthetical data are cases for which no LC/TOF response was observed).

MPB			**MTD**			**TRM**			**ZPD**		
	TOF +	**TOF –**		**TOF +**	**TOF -**		**TOF +**	**TOF -**		**TOF +**	**TOF -**
Chip +	257	20 (7)	**Chip +**	146	17 (2)	**Chip +**	136	29 (3)	**Chip +**	104	12 (0)
Chip –	0	1581	**Chip –**	0	1695	**Chip –**	0	1693	**Chip –**	0	1742

Conclusions: The percent agreement between the MBP, MTD, TRM, and ZPD Randox® Drugs of Abuse Custom Array VIII Biochip and an LC/TOF screen were 98.9%, 99.0%, 98.4%, and 99.3%, respectively. The specificity and sensitivity for the

assays were as follows: MBP (100%, 92.8%), MTD (100%, 89.6%), TRM (100%, 82.4%), and ZPD (100%, 89.6%). Based on the LC/TOF responses, had the LC/TOF decision points been more aligned with the Biochip cutoff concentrations, sensitivity would have increased although there may have been some loss of specificity.

Randox® Evidence, Accurate Mass Screeening, Drug-Impaired Driving

K56 Ethylone: Development and Validation of a Quantitative Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Method With Analytical Confirmation in Toxicology Casework

Stephanie Kumor, MA, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090; Joseph Homan, MS, 3701 Welsh Road, Willow Grove, PA 19090; Annette Ervin, BS, 3701 Welsh Road, Willow Grove, PA 19090; Donna M. Papsun, MS, Willow Grove, PA 19030; and Barry K. Logan, PhD, NMS Labs/CFSRE, 3701 Welsh Road, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to discuss the emerging designer drug class of synthetic cathinones with a focus on ethylone, and will be able to undertake the development of an analytical assay using LC/MS/MS for their detection in biological fluids.

This presentation will impact the forensic science community by raising awareness of the abuse and toxicity of ethylone and other novel synthetic cathinones as well as improving their detection and quantitation in biological matrices.

Ethylone is considered a next generation synthetic cathinone since detection in laboratories began in late 2011 and has been steadily increasing. The effects of oral doses of ethylone are reported to be similar to those of cocaine and amphetamine, which include excitation, increased energy, and euphoria. The abuse of designer cathinones has increased in popularity since 2009 and they are often sold as "bath salts" or "plant food," although they are labeled "not for human consumption." Synthetic cathinone products are continually changing with slight structural alterations in order to circumvent drug control regulations of early-generation analogs such as methylone, mephedrone, and MDPV. As this drug is not part of many laboratories' routine testing procedures, there is limited available information on its toxicity and pharmacological effects, which raises a public health concern due to the increase in its abuse. The development of a sensitive and reliable validated method of detection was undertaken to fill this gap.

This assay was developed to detect and quantitate ethylone in addition to four related synthetic cathinones: butylone, pentylone, flephedrone (4-fluoromethcathinone (4-FMC)), and 3-fluoromethcathinone (3-FMC). Whole blood and urine samples were extracted using Solid Phase Extraction (SPE). Analytes were quantified using positive mode LC/MS/MS. Separation was achieved on a BEH C18, 2.1mm x 100mm column with mobile phases consisting of ammonium acetate buffer, pH9, and acetonitrile. The two pairs of structural isomers, ethylone and butylone, and flephedrone and 3-FMC, were separated chromatographically using the high pH-buffered mobile phase.

The optimized method was fully validated according to the Scientific Working Group for Toxicology (SWGTOX) guidelines. Linearity was established from 10ng/mL to 1,000ng/mL using six calibration points. Replicates (n=5) at each concentration level were analyzed and the correlation coefficient was >0.99 for all analytes. All five synthetic cathinones were measured at three different concentrations to give precision ≤10% Coefficient of Variation (CV) and accuracy ±10% for both within- and between-run experiments. For ethylone, the maximum average intra- and inter-run imprecision were 8.1% and 5.1%, respectively. The lower limit of detection for ethylone was 1.25ng/mL. Stability experiments (n=6) indicated that ethylone is stable in blood for up to two days at room temperature and for at least 28 days if kept refrigerated or frozen.

A set of 21 blood subject samples which had screened positive for ethylone were tested using this method. Of the cases with demographic information available, the median age was 26 years, and included 12 males and 5 females. The ethylone results of the 21 blood cases ranged from 7ng/mL to 24,500ng/mL with a mean and median concentration of 1,982ng/mL and 461ng/mL, respectively. Five were positive for ethylone in combination with other designer stimulants (methylone, butylone, flephedrone, and alpha-PVP). It was noted in the case history for the highest value of ethylone detected that the individual experienced excited delirium prior to collapsing. The detection of ethylone and other designer cathinones in these forensic toxicology and postmortem cases demonstrates the need for a reliable quantitative method over a wide concentration range.

The increased use of ethylone and other newer synthetic cathinone analogs required the development of a quantitative analytical assay. The observed concentrations in biological fluids provide insight to forensic toxicology in regard to the toxicity and expected levels of ethylone in casework.

Ethylone, Psychoactive Substances, Validation

K57 Paper Spray Mass Spectrometry for Rapid Drug Screening From Dried Blood Spots

Rachel Potter, BS, 402 N Blackford Street, Indianapolis, IN 46202; and Nick Manicke, 402 N Blackford Street, LD 326E, Indianapolis, IN 46202*

After attending this presentation, attendees will understand the emerging technique of paper spray mass spectrometry and its application in forensic toxicology as an effective, rapid, and simple screening technique for illicit drugs, pharmaceuticals, and their metabolites.

This presentation will impact the forensic science community by providing data demonstrating paper spray's potential to be highly useful to forensic toxicology. As a screening tool, it can effectively expedite and simplify existing procedures for identifying drugs and drug metabolites in biofluids, allowing for higher sample throughput and faster turnaround times.

Paper spray ionization is able to extract analytes and generate gaseous ions directly from dried blood spots and other biofluids at toxicologically relevant concentrations with no sample preparation and minimal solvent usage. Whole blood samples were dried directly onto Whatman™ 31ET Chromatography Paper (although robustness has been proven using other types of paper), which was cut to fit an in-house designed cartridge. With the cartridge placed in front of the inlet to a mass spectrometer, a small amount of solvent (20µL-40µL) was applied to the back of the paper. While most of the large biological molecules are left behind, undissolved in the solvent (typically 95% methanol with 0.01% acetic acid), the soluble analytes are extracted and traveled with the solvent front by capillary action though the paper. Once the solvent had completely wet the paper, a high voltage of 3kV-4kV was applied, inducing an electrospray at the paper's tip. As the charged droplets from the electrospray travel toward the inlet of the mass spectrometer, the solvent evaporates, generating gaseous analyte ions, which then enter the mass spectrometer.

The present study utilized a Thermo Scientific™ TSQ Vantage™ triple quadrupole mass spectrometer operated in Selected Reaction Monitoring (SRM) mode to investigate paper spray's detection limits and selectivity for 154 toxicologically relevant drugs and drug metabolites. Detection limits for the targets were determined by spiking known amounts of the targets into drug-free human blood. Positive identification of a drug or metabolite was achieved when both a qualifier and quantifier SRM transition for the target were present at a predetermined intensity and the ratio between the two transitions was within ±25% of the expected value. The detection limits achieved by direct dried blood spot analysis by paper spray were compared to screening cut-off levels specified by an area toxicology laboratory. These cut-off values ranged from 1ng/mL to 30,000ng/mL, depending on the target compound. Representative analytes spiked into whole blood from the following classes of drugs have already been detected at levels below the desired screening cutoff: anticonvulsants, anesthetics, cocaine and its metabolites, sedatives, benzodiazepines, analgesics, and amphetamines.

Selectivity of the method was assessed by compiling a list from DrugBank and the Human Metabolome Database of drugs and metabolites of drug origin with the same nominal mass as the target compounds. The potential for these compounds to yield false positive results was assessed based on their expected abundance and ionization efficiency relative to the target compound, as well as their MS/MS spectra. In nearly all cases, the selectivity of paper spray MS/MS was adequate. Some cases of interference were identified; however, in nearly all cases, the interferences were closely related structural isomers from the same compound class, such as morphine and hydromorphone.

The data collected on the achievable detection limits and selectivity indicate that paper spray is a promising method for quickly screening for a large number of drugs and drug metabolites in blood.

Paper Spray Mass Spectrometry, Toxicology, Dried Blood Spots

K58 Application of Mixed-Mode Ultra High-Performance Liquid Chromatography to the Analysis of Drugs in Urine

Ira S. Lurie, PhD, George Washington University, Dept of Forensic Science, 2100 Foxhall Road, NW, Somers Hall, Lower Level, Washington, DC 20007; Cassandra Lee Clyde, MFS, Office of the Chief Medical Examiner, 401 E Street, SW, 6th Fl, Washington, DC 20024; Samantha A. Blake, MFS, Tucson Police Department Crime Laboratory, 1306 W Miracle Mile, Tucson, AZ 85705; Stacey L. Obrien, BS, 7406 Oriole Avenue, Springfield, VA 22150; and Ihuoma A. Igwilo, MBBS, George Washington University, Dept of Forensic Sciences, 2100 Foxhall Road, NW, Somers Hall L10, Washington, DC 20007*

The goal of this presentation is to present a novel approach for the analysis of drugs in urine which utilizes a single column for both the reversed phase chromatographic and hydrophilic interaction chromatographic separation of the target solutes. Attendees will learn how the proposed methodology reduces ion suppression or ion enhancement due to solute co-elution or biological matrix effects, increases accuracy of solute identification, and minimizes sample preparation time and ample analysis time.

This presentation will impact the forensic science community by describing how the proposed methodology provides practical advantages over existing methodology in terms of rapid sample cleanup in tandem with increased resolution of target solutes without the need to change columns or instruments.

For the first time, methodology is presented for the analysis of drugs in urine employing a single column and the same elution solvents at different ratios for orthogonal separations. Depending on the elution solvent blend, separations for the basic drugs in the SAMHSA-5 panel in urine could be carried out both in the Reversed Phase Chromatographic (RPC) and Hydrophilic Interaction Liquid Chromatographic (HILIC) modes. For the analysis of the drugs in urine, all solutes could be separated using a combination of both chromatographic systems; this minimized ion suppression and allowed the unique identification of each solute using retention time. For both separations a 2.1mm x 150mm x 2.7µm superficially porous dimethylpentafluorophenylpropyl (PFP) column was employed using combinations of two acetonitrile-water-ammonium fomate elution solvents with Time-Of-Flight/Mass Spectrometric (TOF/MS) analysis.

For the separation of amphetamine, methamphetamine, MDA, MDMA, MDEA, morphine, codeine, 6-monoacetylmorphine, benzoylecgonine, and PCP, orthogonal separations were obtained using RPC and HILIC (R^2=0.0839 for relative retention time). For the RPC separation mode, a 12-minute gradient was performed, while a 6-minute isocratic separation was performed for the HILIC mode. Solid Phase Extraction (SPE) was performed on a mixed mode MM1 column. The solutes of interest were successfully separated with good recovery and allowed for minimum ion suppression or ion enhancement for the Ultra High-Performance Liquid Chromatography (UHPLC) TOF/MS analysis. For the SPE sample preparation, no evaporation and reconstitution step was required, due to the fact that the elution solvent was directly compatible with both the HILIC and RPC analysis on the PFP column. For most solutes, using both chromatographic modes linearity was observed over at least two orders of magnitude with R^2=≥0.992. For the one outlier amphetamine (HILIC), linearity is observed over two orders of magnitude using RPC with R^2=0.998. In addition, the limit of quantification for most solutes was adequate for both screening and confirmatory test cutoff concentrations, while the limit of detection was adequate for O6-monoacetylmorphine.

The applicability of the above chromatographic approach for the complementary separation of synthetic cathinones ("bath salts") and pain management drugs will be demonstrated.

Drugs, Urine, Liquid Chromatography

K59 Development and Validation of Two Methods for the Analysis of Synthetic Cannabinoids in Whole Blood

Marykathryn Tynon, MSFS, 3701 Welsh Road, Willow Grove, PA 19090; Joseph Homan, MS, 3701 Welsh Road, Willow Grove, PA 19090; Sherri L. Kacinko, PhD, 3701 Welsh Road, Willow Grove, PA 19090; and Barry K. Logan, PhD, NMS Labs/CFSRE, 3701 Welsh Road, Willow Grove, PA 19090*

After attending this presentation, attendees will better understand the Scientific Working Group for Forensic Toxicology (SWGTOX) -compliant approach to method validation for the analysis of two classes of synthetic cannabinoid compounds in forensic whole blood samples using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) technology.

This presentation will impact the forensic science community by providing information about two complementary analytical methods used to analyze 34 synthetic cannabinoid compounds of the Indazole Carboxamide (NACA) and Indole classes, many of which have only recently appeared on the market. This presentation will also inform attendees about recent trends in positivity rates for these compounds in forensic samples.

Since the mid to late 2000s, an increasing number of New Psychoactive Substances (NPS), including synthetic cannabinoids, have been appearing on the illicit drug market in the United States. In 2011, one such compound, AM-2201 was ranked at number 21 in the National Forensic Laboratory Information System (NFLIS) ranking of most frequently encountered drugs in the nation's crime laboratories. In the same year, looking in the hallucinogen category, seven unique synthetic cannabinoids appeared in the top 16 rankings. By 2013, there were 17,242 synthetic cannabinoids cases in the United States. In 2014, XLR-11 was joined by a new category of NACA compounds including AB-FUBINACA,AB-PINACA, ADBICA, 5F-ADBICA, ADB-PINACA, ADB-FUBINACA, 5F-ADB-PINACA, 5F-APICA, PB-22, 5F-PB-22, BB-22, FUBIMINA, THJ-018, 5F-AB-001, AB-PINACA AB-CHMINACA, and ADB-CHMINACA, in addition to the more traditional indole class members including the JWH, and AM series, and XLR-11.

This presentation describes two complementary methods for the analysis of 34 currently popular synthetic cannabinoids compounds in whole blood using Liquid-Liquid Extraction (LLE) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). The compounds were segregated into two groups depending on their chemistry (NACA and Indole classes) for analyses, which were individually optimized for extraction conditions and performance. The cutoff concentration for the NACA compounds in the NACA group were 1ng/mL for AB-FUBINACA, ADBICA, 5F-ADBICA, ADB-FUBINACA, 5F-ADB-PINACA, and AB-CHMINACA and 0.2ng/mL for ADB-PINACA, AB-PINACA, and ADB-CHMINACA. The cutoff concentration for the Indole compounds were 0.1ng/mL for JWH-018, AM-2201, JWH-122, JWH-081, MN-18, 5F-MN-18, MN-25, FUB-PB-22, MDMB-CHMINACA, and AB-001 and 0.2 ng/mL for JWH-210, UR-144, XLR-11, FUB-AKB48, and APICA and 1ng/mL for 5F-APICA. Since this is a qualitative test, any samples with analyte about the cutoff concentration are considered positive. The methods were subject to a SWGTOX-compliant validation procedure that evaluated precision around the decision concentration (cut-off) , stability in matrix and on-instrument, sensitivity and specificity, robustness, an evaluation of interfering compounds, matrix effect, and extraction efficiency. After the validation was complete, authentic patient samples were analyzed using the method and positivity trends were evaluated.

Sample preparation consisted of single-step LLEs using 3mL Methyl Tertiary Butyl Ether (MTBE) for the NACA group (*n*=9) and 3mL 99% Hexane/1% Ethyl Acetate for the Indole group (*n*=25). The analytical method consisted of separation using an ACQUITY® UPLC BEH C18 (100mm x 2.1mm, 1.7-micron) column coupled with a VanGuard™ BEH C18 1.7-micron guard column and a gradient elution. The NACA class started with an initial mixture of 55% mobile phase A (0.1% formic acid in water) and 45% mobile phase B (80% acetonitrile/20% methanol), while the Indole class started with an initial mixture of 50% mobile phase A and 50% mobile phase B, both transitioned to a final mixture of 5% mobile phase A and 95% mobile phase B. Both NACA and Indole classes had a total runtime of eight minutes. Both methods were run on a Waters® Xevo-TQS®.

This method produced data that met the acceptance criteria for precision around the cutoff concentration and was shown to be 100% sensitive and specific in blinded spiked controls in diverse whole blood samples. The method was also shown to meet validation criteria for precision around the decision concentration, stability in matrix and on instrument, robustness, interference, matrix effect, and extraction efficiency.

Subsequently, the method has been in production for four months, during which time a total of 661 samples have been tested. Switching from the previous scope to the scope described in this presentation, the positivity rate for the test increased from approximately 7% on the prior method to 35% on the method described herein. The most prevalent compounds in casework samples during the period March-June 2015 were AB-CHIMINACA, XLR-11, and ADB-CHMINACA.

Over the four months the method has been in place, the number of positives has demonstrated a decline, suggesting further evolution of the synthetic cannabinoid illicit market. The laboratory is currently validating a revised scope developed using various drug intelligence sources and has committed to a nine-month update on the scope of testing to keep pace with the changing market.

Synthetic, Forensic Toxicology, Cannabinoids

K60 A Two-Year Comparative Analysis of Novel Psychoactive Substances (NPS) Detected in Blood, Urine, and/or Oral Fluid in Attendees at an Electronic Dance Music (EDM) Festival

Amanda L.A. Mohr, MSFS, Center for Forensic Science, Research & Education, 2300 Stratford Avenue, Willow Grove, PA 19090; Jillian K. Yeakel, MS, 3864 Courtney Street, Ste 150, Bethlehem, PA 18017; Melissa Friscia, MSFS, 429 Grand Avenue, Langhorne, PA 19047; Francis X. Diamond, BS, 3701 Welsh Road, Willow Grove, PA 19090; and Barry K. Logan, PhD, NMS Labs/CFSRE, 3701 Welsh Road, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to assess and review trends in recreational drug use, specifically related to NPS from self-reported drug-use data and analytical results from the analysis of blood, urine, and oral fluid collected over a two-year period within an EDM population.

This presentation will impact the forensic science community by providing data on the temporal trends of NPS use within this population and evaluate the diversity and nature of emerging analytes. Attendees will also be able to evaluate the utility of blood, urine, and/or oral fluid for detecting NPS drug use.

The use of NPS at EDM festivals is widely documented by surveys with festival attendees, reflected in online discussions groups associated with EDM culture and recently has become a focus of media attention due to several hospitalizations and deaths attributed to NPS use at these events. The use of these novel and potentially toxic drugs within these venues makes EDM festivals an important site to collect information regarding recreational drug use and potentially characterize emerging analytes.

Participants were recruited during an EDM festival in Florida over a two-year period. The study received institutional review approval for human subject studies. After obtaining informed consent, each participant filled out a brief questionnaire regarding prescription medication and recreational drug use within the past week. Participants were asked to provide a blood, urine, and an oral fluid sample for laboratory-based drug screening and confirmation.

Over the course of two years, 396 EDM attendees participated in the study. The average age of the study participants was 22 years. Not all subjects provided all three biological samples. During the two-year sample collection period, 126 blood samples, 226 urine samples, and 330 oral fluid samples were provided. Blood, urine, and oral fluid samples were screened using Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS) via the Waters® ACQUITY® UPLC Iclass Xevo® G2-S QTof, in addition to other techniques. Specimens were also tested for alcohol. Any sample which screened positive was sent for confirmation.

When asked whether or not the individual had taken any medicinal or recreational drugs within the past week, in both 2014 and 2015, 72% percent of the participants answered "yes." The percentage of participants who self-reported using "Molly," MDMA, or Ecstasy within the last week was similar for both years (21% in 2014 and 14% in 2015). Twenty-five blood samples (42%) from the 2015 data set screened positive for a common drug of abuse/metabolite or NPS compared to 58% in 2014. Of the blood samples that screened positive, the detection of at least one NPS decreased from 55% in 2014 to 33% in 2015. A similar trend was seen with the urine and oral fluid samples where there was a decrease in positivity for NPS, which went from 33% in 2014 to 18% in 2015 for urine and from 32% to 18% for oral fluid samples.

During 2014, the most commonly detected NPS was α-Pyrrolidinopentiophenone (alpha-PVP), which was followed by methylone and dimethylone; however, in the 2015 data set, there was a decrease in the number and diversity of NPS drugs detected. The fact that in 2015 alpha-PVP was not detected in any of the blood samples was a surprising finding given that it was the most commonly encountered NPS in 2014 and there have been increasing reports of its prevalence within the state of Florida. Conversely, more samples (blood, urine, and oral fluid) were confirmed positive for MDMA in 2015 than in 2014.

This comparative analysis provides the first temporal data related to NPS use within the United States at EDM festivals. With respect to NPS, there was a high turnover rate in the prevalence of specific drugs from year to year. This suggests EDM festivals represent important study populations that can be used to garner drug intelligence information with respect to what emerging compounds are currently being abused, which in turn can help guide forensic professionals in providing an updated and comprehensive scope for NPS testing.

NPS, Trends, EDM Festivals

K61 Metabolic Profile Determination of NBOMe Compounds Using Human Liver Microsomes

Keith-Dane H. Temporal, BS, 1024 Riverside Drive, Philadelphia, PA 19154; Melissa Friscia, MSFS, 429 Grand Avenue, Langhorne, PA 19047; Karen S. Scott, PhD, Arcadia University, 450 S Easton Road, Glenside, PA 19038; Amanda L.A. Mohr, MSFS, Center for Forensic Science, Research & Education, 2300 Stratford Avenue, Willow Grove, PA 19090; and Barry K. Logan, PhD, NMS Labs/CFSRE, 3701 Welsh Road, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to assess and review the proposed *in vitro* metabolic profile of members of the hallucinogenic NBOMe series, including 25I-NBOMe, 25H-NBOMe, 25C-NBOMe, and 25B-NBOMe.

This presentation will impact the forensic science community by providing information on the identities of unique metabolites for the various NBOMe compounds that can be added to analytical protocols for biological markers of their ingestion and increase the likelihood of detection of their use.

In recent years, a new group of emerging psychoactive substances known as NBOMes have gained popularity with recreational drug users as an alternative to the classic hallucinogen Lysergic Acid Diethylamide (LSD-25). NBOMes are derivatives of a group of substituted phenethylamines known as 2C compounds, which were first pioneered by Alexander Shulgin in the 1980s. These stimulant-type drugs act on the 5-HT 2A serotonin receptors resulting in a constellation of psychedelic and hallucinogenic effects. The NBOMe name describes the N-methoxybenzyl substitution for corresponding 2C compounds, such as 25I-NBOMe (2-(4-iodo-2,5-dimethoxyphenyl)-N-((2-methoxyphenyl)methyl)ethanamine), which is derived from 2C-I (2,5-Dimethoxy-4-iodophenethylamine). Other NBOMe substitutions of the various existing 2C compounds, such as 25B-NBOMe, 25H-NBOMe, and 25C-NBOMe, have also been available to recreational drug users by clandestine laboratories making and distributing such compounds as "research chemicals."

Administration of this substance occurs in the forms of blotter paper dosages, powders, and liquid formulations. The NBOMe substitution is suggested to increase the potency and activity of these substances relative to the 2C compounds. This is problematic for users of NBOMes who often mistake the drug for LSD because recommended dosages for its desired effects are overestimated and can result in acute toxicity. NBOMes' adverse effects include bouts of violent tendencies, paranoia, episodes of intense psycho-stimulation, and fatal intoxications.

The goal of this project was to identify the main metabolites of various NBOMe compounds using *in vitro* incubation with human liver microsomes and ultimately to confirm these findings in authentic forensic specimens.

Aqueous standards containing the drugs of interest were incubated with human liver microsome preparations (20mg/mL, 50-individual pool) and an NADPH generating system. The mixtures were incubated for two hours at 37°C, then stopped with acetonitrile. Aliquots of the reaction mixture were extracted after centrifugation at 10,000rpm, brief evaporation of the acetonitrile from the supernatant, and final centrifugation at 10,000rpm with a centrifugal filter. Extracts were then analyzed by Liquid Chromatography Time-Of-Flight Mass Spectrometry (LC/qTOF/MS) using a Waters® ACQUITY® UPLC Iclass Xevo® G2-S QTof. Data analysis was performed using the Waters® Forensic Toxicology Application Solution with UNIFI™ 1.7.

The Ultra Performance Liquid Chromatography (UPLC) separation was accomplished using an ACQUITY®UPLC® BEH C18 column (2.1mm x 150 mm, 1.8µm) with a column temperature of 50oC and a gradient elution method. Mobile phase A was 5mM ammonium formation, pH3, and mobile phase B was 0.1% formic acid in acetonitrile. The MS data was obtained using electrospray ionization in positive ion mode, with a scan range of 50m/z-1,000m/z. Data analysis was performed using UNIFI™.

The primary metabolite identified for 25I-NBOMe following this incubation method was its N-demethoxybenzylated form, also known as 2C-I. Other phase I metabolites of 25I-NBOMe yielded O-demethylated and several demethoxylated isomers of the parent compound. 25H-NBOMe (or 2-(2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine) showed similar metabolic fate, including identification of its 2C analog, 2C-H (2-(2,5- dimethoxyphenyl)ethanamine), and a hydroxylated form. 25B-NBOMe (2-(4-bromo-2,5-dimethoxyphenyl)-N-((2-methoxyphenyl)methyl)ethanamine) showed at least four unique metabolites, including a debrominated O-demethylated hydroxylated form. Other metabolic transformations observed included dehydrogenation, demethylation, and hydroxylation of the parent compound. The primary phase I metabolite changes to the NBOMe class include O-demethylation at the three methoxy groups, single and *bis*-hydroxylations, and cleavage at the amine (N-demethoxybenzylation).

In conclusion, successful *in vitro* metabolism of NBOMes with human liver microsomes and subsequent analysis of metabolites using a high mass accuracy method with LC/qTOF provided confident identification for the primary metabolites by Cytochromes P450 (CYP) enzymes. The results of this project will positively impact the ongoing research and efforts to identify biological markers of use for NBOMes as they gain prevalence among recreational drug users and are seen with increasing prevalence within forensic settings.

NBOMes, Metabolism, QTOF

K62 Case Report of AB-FUBINACA Exposure With Chemical and Toxicological Confirmation

David Buzby, BS, 3102 Georgetown Road, Cinnaminson, NJ 08077; Donna M. Papsun, MS, Willow Grove, PA 19030; Mark Nyvean, MD, Hartford Hospital, University of Connecticut School of Medicine, 80 Seymour Street, Hartford, CT 06102; and Barry K. Logan, PhD, NMS Labs/CFSRE, 3701 Welsh Road, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to implement lessons learned from the combined efforts of many groups in the successful identification of a group intoxication event posing a threat to public health and safety.

This presentation will impact the forensic science community by introducing a case series of individuals presenting to a hospital emergency department after exposure to the third-generation synthetic cannabinoid AB-FUBINACA with symptoms ranging in severity.

A number of young adults were attending a party at which a variety of recreational substances were being used. During the course of the event, nearly a dozen individuals fell ill and emergency services were called. A range of symptoms were reported, including sedation, seizures, and one case of ventricular fibrillation. Four patients who were experiencing adverse effects all related having taken a clear capsule containing a white powder, which was sold to them as "Molly."

During a subsequent police investigation, drug materials containing the following substances were seized: 5-MeO-DMT (known as "foxy-methoxy"), 5-MeO-MiPT, Dimethyl Tryptamine (DMT), escitalopram, alprazolam, 4-Acetyl-DMT, psilocin, 1-(benzofuran-5-yl)-N-methylpropan-2-amine (MAPB), and AB-FUBINACA. These substances range in their pharmacological properties and would cause a variety of physiological and psychological effects. Some of these substances are tryptamine derivatives, while escitalopram and alprazolam are prescription medications that may be abused. MAPB is a benzofuran drug with stimulant properties, and AB-FUBINACA is a synthetic cannabinoid agonist. All four individuals admitted to taking "Molly," which is often a street name for MDMA; however, other substances such as methylone or BZP have been sold under the same name.

Urine was collected from four individuals (all male) presenting to the emergency room after taking the described pill and falling ill. Hospital testing of the urine was limited to testing for the presence of MDMA using immunoassay. After initial negative screening results, the samples were then submitted to a reference laboratory for comprehensive toxicology testing.

An escalating approach was taken for this toxicological investigation and used a variety of methodologies. Initial testing was requested for DMT, with negative results. Testing was expanded to include a hallucinogenic screen using **Liquid Chromatography**/Time-Of-Flight/Mass Spectrometry (LC/TOF/MS) and Gas Chromatography/Mass Spectrometry (GC/MS) with spectral deconvolution software (DRS) using an expansive in-house database including a large number of novel psychoactive substances including 5-MeO-DMT, 5-MeO-MiPT, DMT, escitalopram, alprazolam, 4-Acetyl-DMT, psilocin, and MAPB. After these two screens were completed, there were no significant findings and no findings that tied the four cases together. Positive findings were consistent with either valid prescriptions or medications administered in the hospital. Finally, urine sample testing was performed for a range of 12 synthetic cannabinoid metabolites including AB-PINACA N-Pentanoic Acid, ADBICA N-Pentanoic Acid, AB-FUBINACA Butanoic Acid, ADB-PINACA, 5-Fluoro-PB-22 carboxyindole, JWH-073 N-Butanoic acid, PB-22 3-carboxyindole, BB-22 3-carboxyindole, AB-CHMINACA 3-methyl-butanoic acid, JWH-018 N-pentanoic acid, AKB48 N-pentanoic acid, and UR-144 N-pentanoic acid. Testing was conducted using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). Testing of the four urine samples identified the presence of AB-FUBINACA Butanoic acid in all samples, although in one case the concentration was only 22% of the cut-off (5.0ng/mL); however, all other acceptance criteria were met. There was no response for transitions for AB-FUBINACA Butanoic acid in any of the blank urine samples. Toxicology testing confirmed the use of AB-FUBINACA, which was consistent with the results of chemical analysis of a capsule recovered from the scene, consistent with that described by the four individuals tested. AB-PINACA N-pentanoic acid was also detected in three of the four cases, suggesting prior use of AB-PINACA .

This case highlights the joint efforts of emergency responders, poison control, medical doctors, toxicologists, investigators, and forensic chemists in order to successfully identify the new and dangerous synthetic cannabinoid agonist, AB-FUBINACA, as the responsible agent in a series of intoxications. This also highlights the threat to recreational drug users who are sold counterfeit or compromised substances, as in this case, where AB-FUBINACA was sold as "Molly."

Synthetic Cannabinoids, AB-FUBINACA, Molly

K63 Cannabinoid Receptor Bioassay: A Characterization of UR-144, XLR-11, and Their Metabolites and Degradants

Kelsey Longe, BS, Marshall University Forensic Science Program, 1401 Forensic Science Drive, Huntington, WV 25701; Amy B. Cadwallader, PhD, Aegis Sciences Corporation, 365 Great Circle Road, Nashville, TN 37228; Darcie Wallace-Duckworth, PhD, 515 Great Circle Road, Nashville, TN 37228-1310; and Pamela J. Staton, PhD, Marshall University Forensic Science MSFS & Center, 1401 Forensic Science Drive, Huntington, WV 25701*

After attending this presentation, attendees will better understand the cannabimimetic nature of UR-144, XLR-11, and their associated metabolites and degradants.

This presentation will impact the forensic science community by providing supporting evidence for the continued scheduling of UR-144, XLR-11, and some of their associated degradants and metabolites.

Synthetic cannabinoids, one of the largest-growing and widely varying groups of designer drugs, have become popular in recent years due to the cannabimimetic high they offer to users.[1] The similarities in the effects of synthetic cannabinoids and marijuana (Δ^9-tetrahydrocannabinol) are thought to be the result of these compounds interacting with the same G Protein-Coupled Receptors (GPCRs).[2] These GPCRs are more commonly referred to as the cannabinoid binding receptors CB1 and CB2 and are located in the body's central and peripheral nervous systems, respectively. Due to their separate locations, CB1 receptors are generally associated with the hallucinogenic effects of cannabinoids, while the CB2 receptors are linked to the therapeutic effects of cannabinoids; however, very little has been discovered regarding the potencies of these compounds at these receptors.[3] This lack of information regarding the cannabimimetic nature of these drugs makes it difficult for authorities to schedule them.

In order to learn more about how different synthetic cannabinoids interact with the CB1 and CB2 receptors, the potency (EC_{50}) of two of these synthetic cannabinoids, UR-144 and XLR-11, as well as ten of their metabolites and degradants, was investigated using a mammalian cell-based cannabinoid receptor bioassay. For UR-144, EC_{50} values of 8.5ng/mL and 3.6ng/mL were found for the CB1 and CB2 receptors, respectively. Two of the remaining UR-144 compounds, the UR-144 degradant and the N-(2-hydroxypentyl) metabolite, were determined to be more potent at the CB1 receptors, while the N-(4-hydroxypentyl) and N-(5-hydroxypentyl) metabolites both were found to be more potent than UR-144 at the CB2 receptors. With XLR-11, the CB1 and CB2 EC_{50} values were found to be 101ng/mL and 6.6ng/mL, respectively. All three XLR-11 metabolites and degradants tested proved to be more potent than XLR-11 at the CB2 receptors, with one of these three compounds being more potent at the CB1 receptors as well. Combining the knowledge that seven of the ten metabolized and degraded forms of UR-144 and XLR-11 tested demonstrated greater potencies than the parent compounds, and the fact that the metabolized and degraded forms are likely to be more commonly seen in forensic toxicological samples than UR-144 and XLR-11 themselves, it can be suggested that the bioassay shows great potential as a screening method for toxicological samples.

In conclusion, this study's results support the claim that several of the UR-144 and XLR-11 compounds are cannabimimetic due to their activity with the CB1 and CB2 receptors. This data is not only applicable to forensics by helping determine if these drugs should continue to be scheduled, but it can also be useful to the field of medicinal chemistry in which cannabinoids with a greater potency at the CB2 receptors than the CB1 receptors are being investigated as potential therapeutic treatments.[4]

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UR-144, XLR-11, Cannabinoid Binding Receptors

K64 Analysis for Synthetic Cannabinoids in Oral Fluid Samples Obtained From a Music Festival Cohort

Marykathryn Tynon, MSFS, 3701 Welsh Road, Willow Grove, PA 19090; Joseph Homan, MS, 3701 Welsh Road, Willow Grove, PA 19090; Sherri L. Kacinko, PhD, 3701 Welsh Road, Willow Grove, PA 19090; and Barry K. Logan, PhD, NMS Labs/CFSRE, 3701 Welsh Road, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to develop and validate an analytical method for the analysis of synthetic cannabinoid compounds in oral fluid samples using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) technology and evaluate the findings from testing a cohort of subjects with high rates of Novel Psychoactive Substance (NPS) use for synthetic cannabinoids.

This presentation will impact the forensic science community by advancing the science of oral fluid testing for novel psychoactive substances and raising awareness of the latest trends in a class of potentially dangerous drugs.

Oral fluid has many advantages as a toxicological specimen, including ease of collection, which can be done in the open and without the need for privacy or a same-gender collection agent. The specimen can be collected close in time to any alleged drug impairment, without the need for transport and the associated phlebotomy, law enforcement, and medical professionals' time. In a workplace setting, the rapid collection also reduces the amount of time an employee is away from their workstation, reducing the employers' costs. From the laboratory perspective, the sample is less complex than blood or urine, simplifying sample preparation. Oral fluid sample analysis is not without its challenges, in that drug concentrations, especially for acidic compounds, may be below those in blood, sample volume is limited, and there is less data supporting correlation of oral fluid drug concentrations to likelihood of impairment than exists even for blood. Synthetic cannabinoid drugs have increasingly been implicated in motor vehicle collisions and are a growing concern in the workplace due to the fact that there is currently no routine testing for these drugs. The objective of this presentation is to describe the development and validation of a test for 27 commonly encountered synthetic cannabinoid drugs in oral fluid and the application of the test to samples collected from a drug-using cohort.

The matrix for the test was oral fluid (~1mL) mixed with 3mL of a preserving buffer containing salts and isopropanol (Quantisal®). Sample preparation was performed using Solid Phase Extraction (SPE) utilizing Oasis® HLB 60mg extraction columns. The samples were washed using deionized water, 1M ammonium carbonate buffer pH10, and hexane before elution with acetonitrile. Analysis was performed by LC/MS/MS. The method was designed to detect JWH-018, AM-2201, JWH-122, JWH-210, JWH-081, UR-144, XLR-11, AB-FUBINACA, ADBICA, 5F-ADBICA, ADB-PINACA, ADB-FUBINACA, 5F-ADB-PINACA, JWH-018 adamantyl, 5F-JWH-018 adamantyl carboxamide, JWH-018 adamantyl carboxamide, PB-22, AKB-48, 5F-AKB-48, BB-22, AM-2201 benzimidazole, THJ-2201, THJ-018, 5F-AB-001, AB-PINACA, and AB-CHMINACA. The analytical method consisted of separation using an ACQUITY® UPLC® BEH C18 (100mm x 2.1mm, 1.7-micron) column coupled with a VanGuard BEH C18 1.7-micron guard column and a gradient elution. An initial mixture of 55% mobile phase A (0.1% formic acid in water) and 45% mobile phase B (acetonitrile) was decreased to a final mixture of 5% mobile phase A, 95% mobile phase B. The total run time for the method was 6.0min on a Waters® Xevo-TQS.

Once optimized, the method was evaluated for examined precision around the decision concentration (cut-off), stability in matrix and on instrument, sensitivity and specificity, robustness, an evaluation of interfering compounds, matrix effect, and extraction efficiency. The method produced data that met the acceptance criteria for precision around the cut-off concentration and was shown to be 100% sensitive and specific in blinded spiked controls in diverse oral fluid samples mixed with the preserving buffer. The method was also shown to meet validation criteria for precision around the decision concentration, stability in matrix and on instrument, robustness, interference, matrix effect, and extraction efficiency. After the validation was completed, authentic subject samples were tested.

The samples were collected during an electronic dance music festival in Miami, FL, in March of 2015. Previous studies conducted by the same group have shown drug use rates of 60%-70% in this population, including use of novel psychoactive stimulants.

Synthetic Cannabinoids, Oral Fluid, Forensic Toxicology

K65 Postmortem Findings in Deaths Related to Synthetic Cannabinoids

Robert Kronstrand, PhD, National Board of Forensic Medicine, Dept of Forensic Toxicology, Artillerigatan 12, Linkoping SE 587 58, SWEDEN*

After attending this presentation, attendees will be able to describe and categorize the different groups of synthetic cannabinoids based on their structures, as well as recognize the diversity of synthetic cannabinoids and identify postmortem signs and findings from synthetic cannabinoid intake.

This presentation will impact the forensic science community by adding comprehensive postmortem and toxicological data from a series of synthetic cannabinoid-related deaths.

Background: Synthetic cannabinoids have been in existence for 30 or more years but rather recently, they have emerged among the new psychoactive substances used as recreational drugs and are being encountered in forensic toxicology case work in both the living and the deceased. Even though the synthetic cannabinoids share one mechanism of action with cannabis itself, there seems to be side effects not encountered after smoking cannabis. Acute kidney failure as well as cardiovascular complications from the smoking of synthetic cannabinoids has been reported. In addition to reports of non-fatal intoxications from synthetic cannabinoids, there have been a number of reports of deaths during recent years; however, the pathology and toxicology is not well understood. During the autumn of 2014, Sweden experienced a series of intoxications from synthetic cannabinoids, some of which resulted in death.

Goal: The goal of this study was to closely investigate the postmortem findings and circumstances of the deaths in which a synthetic cannabinoid had contributed to death.

Methods: The study population consisted of all autopsy cases in which the analysis of synthetic cannabinoids had been requested during 2014. The analysis was based on an Ultra High-Performance Liquid Chromatography/quadrupole-Time-Of-Flight (UHPLC/qTOF) method that included 107 analytes and was performed on postmortem femoral blood. The thresholds for positive result were between 0.1ng/g and 0.2ng/g. In some cases, a quantification of the analytes was performed using Ultra High-Performance Liquid Chromatography/Tandem Mass Spectrometry (UHPLC/MS/MS) and compared to data from living recreational users.

Results: In total, 134 cases were screened and 24 of those were positive for one or several synthetic cannabinoids. The synthetic cannabinoids found were BB-22, AB-FUBINACA, THJ-018, THJ-2201, 5F-PB-22, AKB-48, FUB-AKB-48, AB-CHMINACA, FUB-AMB, and MMB-CHMINACA.

In seven cases, synthetic cannabinoids were considered the cause of death or a contributing factor to death. The deaths occurred from August 2 to December 12, 2014. The deceased were all males between 18 years and 56 years of age. All deaths except one were unwitnessed. The autopsy findings were generally few with unspecific findings of pulmonary congestion (three cases), lung emphysema (four cases), and one case with bronchopneumonia. The lungs weights were high (1,214-1,928 grams with a median of 1,348 grams) compared to normal cases (<1,100).

In two cases, the only toxicological finding in femoral blood was MMB-CHMINACA, but in the other five cases, other drugs of abuse, medications, or ethanol were detected. The MMB-CHMINACA concentrations were 1.0ng/g and 3.0ng/g and in the same range as those of recreational users.

Conclusion: In 18% of the suspected cases, one or several synthetic cannabinoids were detected and in 30% of those cases, the medical examiner considered synthetic cannabinoids as the cause of death or that it had contributed to death. There were few specific postmortem findings that could explain the deaths and the concentrations found were not extraordinary. The synthetic cannabinoids seem unpredictably toxic and the mechanisms for their toxicity remain unclear.

Synthetic Cannabinoids, Spice, Psychoactive Substances

K66 Report of Increasing Acetyl Fentanyl Deaths in Allegheny County, Pennsylvania

Todd M. Luckasevic, DO, Allegheny County ME, 1520 Penn Avenue, Pittsburgh, PA 15222; Jennifer K. Janssen, MS, Allegheny County OME, Forensic Laboratory Division, 1520 Penn Avenue, Pittsburgh, PA 15222; Abdulrezak M. Shakir, MD, Alleghany County OME, 1520 Penn Avenue, Pittsburgh, PA 15222; Karl E. Williams, MD, Allegheny County OME, 1520 Penn Avenue, Pittsburgh, PA 15222; and Jessica B. Dwyer, MD, Allegheny County Medical Examiner's Office, 1520 Penn Avenue, Pittsburgh, PA 15222*

After attending this presentation, attendees will learn of a recently detected trend of acetyl fentanyl-related overdose deaths in Allegheny County, PA.

This presentation will impact the forensic science community by recognizing and defining a new synthetic drug, acetyl fentanyl, and illustrating its increased lethality among even seasoned opioid users.

Acetyl fentanyl, N-(1-phenethylpiperidin-4-yl)-N-phenylacetamide and the N-acetyl analog of fentanyl, is a recently developed synthetic opioid with a potency estimated to be 5 to 15 times that of heroin, roughly 16 times that of morphine, and about 3 times less than that of fentanyl. Acetyl fentanyl is currently a Schedule I controlled substance with no recognized medical uses, and is therefore not prescribed by physicians, is not Food and Drug Administration (FDA) -approved, and is not commercially available. Unfortunately, acetyl fentanyl gains access to the public when it is mixed and packaged in drugs marketed as heroin or other opioids. In 2013, the drug gained notoriety when 14 overdose deaths in Rhode Island were attributed to acetyl fentanyl. Following this sentinel report, additional acetyl fentanyl overdose fatalities were reported in Pennsylvania, Louisiana, and North Carolina, further corroborating the possibility of an emerging acetyl fentanyl epidemic. It has since become regular practice in some Pennsylvania coroner's and medical examiner's offices to screen specifically for fentanyl and acetyl fentanyl in cases of apparent opioid-related deaths. Interestingly, this practice has revealed a recently increased number of acetyl fentanyl deaths in Allegheny County, PA.

Since January 2015, the Allegheny County Medical Examiner's Office has identified eight acetyl fentanyl-related deaths. A thorough investigation of the case histories, autopsy, and toxicological findings was conducted. Drug screening was performed on postmortem blood specimens from all eight cases using an **Enzyme-Linked Immuno-Sorbent Assay** (ELISA) five-drug panel (opiates, cocaine, benzodiazepines, oxycodone, and fentanyl) and Gas Chromatography/Mass Spectrometry (GC/MS). Drug screening was also performed on postmortem urine specimens, when available, using GC/MS. Quantitation of acetyl fentanyl was performed on postmortem blood specimens using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) at an outside reference laboratory.

Among the cohort of eight cases, two deaths occurred in March (11 days apart), one in April, two in May (17 days apart), and three in June (within a span of 5 days). All of the cases occurred in sporadic areas of the county with no evident geographic trend. There were a total of six men (75%) and two women (25%), with an age range of 24 years to 57 years (mean 37.8 years). All individuals were White, and seven (88%) had a history of substance abuse. Seven cases (88%) had drug paraphernalia at the scene, including stamp bags (six cases; 75%), syringes (six cases; 75%), and glass pipes (two cases; 25%). Two of the six cases with stamp bags at the scene (33%) had bags labeled with the same name. Postmortem blood specimens from all eight cases screened positive for fentanyl using the ELISA five-drug panel. Using GC/MS, postmortem blood specimens from all eight cases screened positive for acetyl fentanyl, and five (63%) of the eight cases were additionally positive for fentanyl. Postmortem urine specimens were available from seven (88%) of the eight cases. Using GC/MS, all seven urine specimens screened positive for acetyl fentanyl, and four (57%) of the seven cases were additionally positive for fentanyl. Acetyl fentanyl was further quantified using femoral blood in four cases, subclavian blood in one case, and heart blood in three cases. The range, median, mean, and standard deviation for acetyl fentanyl in these eight cases were 2.6ng/mL-2,100ng/mL, 96ng/mL, 443.9ng/mL, and 748.7ng/mL, respectively. Other drugs detected in these cases included morphine, 6-monoacetylmorphine, codeine, cocaine, ethylone, ethanol, diphenhydramine, and clonazepam.

The goals of this report are to highlight the trend of increasing overdose deaths related to acetyl fentanyl and to provide additional evidence to the potential rising epidemic of acetyl fentanyl-related fatalities. Individuals at the forefront of combating drug-related death, including healthcare workers, substance-abuse treatment centers, and forensic services, should be aware of this potent, likely lethal synthetic drug often sold under the disguise of more common street opioids.

Acetyl, Fentanyl, Overdose

K67 The Real Heroin in South Florida: The Detection of a Fentanyl Analog in Postmortem Specimens Using Liquid Chromatography (LC) -Ion Trap Tandem Mass Spectrometry (MS/MS)

Elisa N. Shoff, BS, Miami-Dade Medical Examiner Department, 1851 NW 10th Avenue, Miami, FL 33136; and Diane Boland, PhD, 1851 NW 10th Avenue, Miami, FL 33136*

After attending this presentation, attendees will better understand a new fentanyl analog, ß-hydroxythiofentanyl, that has been linked to eight fatal cases in Miami-Dade County. The detection of this fentanyl analog has previously gone undetected and is presumably being distributed as heroin.

This presentation will impact the forensic science community by providing vital information regarding a new, deadly compound that is complicated to detect using a basic streamlined Gas Chromatography/Mass Spectrometry (GC/MS) blood drug screen.

Fentanyl and its analogs are commonly known as potent synthetic opioids that exhibit powerful and rapid analgesic onset. Fentanyl itself was first introduced into the medical community as an analgesic with a potency approximately 75-125 times that of morphine. The chemical structure of fentanyl allows manufacturers to create analogs that also possess powerful analgesic properties. Although fentanyl and its analogs have legitimate medical use, they are also abused recreationally as interchangeable, substitute, or cutting agents for heroin. Numerous derivatives of fentanyl are sold on the street as synthetic heroin or, more popularly, China White. Due to the potency of fentanyl and its analogs, the abuse of these compounds increases the potential for accidental overdose, especially if consumers are unaware of what they are ingesting. In 2015, the Miami-Dade Medical Examiner Department (MDME) observed an increase in deaths due to fentanyl toxicity (up 300% compared to the previous year) and observed several cases in which the fentanyl analog, β-hydroxythiofentanyl, was implicated in the cause of death. The majority of the cases contain history which indicates heroin use, and none of the deaths reported are related to the abuse of prescription fentanyl.

Approximately 30 medical examiner cases previously screened by GC/MS for the presence of fentanyl and opiates were submitted to a more comprehensive and sensitive screening method. Case blood was extracted via solid-phase extraction using mixed-mode United Chemical Technologies CleanScreen® columns and a positive pressure manifold. Analysis was performed using a Thermo Scientific™ Dionex™ UltiMate® 3000 Ultra High-Performance Liquid Chromatography (UHPLC) coupled to a Bruker amaZon Speed Ion Trap Mass Spectrometer (MS) equipped with Bruker ToxTyper® software. UHPLC separation was achieved over a nine-minute data collection time, using gradient elution on a C18 column (100mm x 2.1mm, 2.2µm) at 40oC using 2mM ammonium formate, 0.1% formic acid, water, and acetonitrile (99:1) mobile phase. Positive mode electrospray ionization MS analysis was performed using UltraScan mode between 70m/z and 800m/z. A custom ToxTyper® acquisition method targeting a panel of analgesics, including, but not limited to, morphine, oxycodone, hydrocodone, heroin, 6-monoacetylmorphine, methadone, buprenorphine, fentanyl, norfentanyl, β-hydroxythiofentanyl, acetylfentanyl, sufentanil, and butyrylfentanyl was utilized. The acquisition method uses an embedded precursor list, which triggers MS/MS and/or MS^3 on the targeted compounds in question, if present in the specimen. β-hydroxythiofentanyl was found to elute at 3.8 minutes with a targeted parent mass of 359m/z. An MS/MS breakdown was performed on the 359 ion, producing a main product ion of 341m/z, indicative of a water loss. The 341 ion was finally introduced to an MS^3 analysis, where a distinct spectral profile was achieved.

Out of the 30 cases analyzed, 10 contained the fentanyl analog, β-hydroxythiofentanyl. Detection of β-hydroxythiofentanyl was confirmed using an in-house library entry created from a certified reference standard, as well as retention time, parent ion, and daughter ion spectra. Two of the cases positive for β-hydroxythiofentanyl had a history of intravenous drug use and were negative for any other compounds, including fentanyl and other opiates. The remaining 8 cases also had a history of intravenous drug use, and the β-hydroxythiofentanyl was detected in addition to heroin and/or fentanyl in the postmortem specimens.

These cases represent some of the first reported β-hydroxythiofentanyl-involved deaths in Florida. The detection of the analog in routine screening procedures using GC/MS presented a problem and required the use of LC/MS analysis to make a definitive identification.

In all cases, it appeared as if norfentanyl was detected in the blood by GC/MS; however, norfentanyl was only confirmed by LC/MS in those cases in which fentanyl was also present. Norfentanyl, in the absence of fentanyl in routine GC/MS analysis, may be an indicator of the presence of β-hydroxythiofentanyl and require further confirmation.

Postmortem, Fentanyl, ß-hydroxythiofentanyl

K68 Blood Clonazepam and 7-Aminoclonazepam Trends in Postmortem and Driving Under the Influence of Drugs (DUID) Cases

Lucas Marshall, MS, Aegis Sciences Corporation, 365 Great Circle Road, Nashville, TN 37228; Timothy A. Robert, PhD, 515 Great Circle Road, Nashville, TN 37228; David L. Black, PhD, Aegis Sciences Corporation, 515 Great Circle Road, Nashville, TN 37228; and Rebecca Heltsley, PhD, 515 Great Circle Road, Nashville, TN 37228*

After attending this presentation, attendees will understand the trends of both parent clonazepam and the primary metabolite/degradant 7-aminoclonazepam in blood from postmortem and DUID cases.

This presentation will impact the forensic science community by illustrating the need to include 7-aminoclonazepam as a marker in the analysis of blood samples to help determine the likelihood of clonazepam exposure.

Clonazepam is a commonly encountered benzodiazepine in postmortem and DUID toxicology. It is routinely prescribed for the treatment of seizure and panic disorders and may also be abused for its sedative, hypnotic, and anxiolytic properties. Often, laboratory analysis of blood samples will only measure the parent analyte clonazepam. Clonazepam is extensively metabolized to 7-aminoclonazepam, an active metabolite, by the reduction of the 7-nitro group. The instability of the 7-nitro group of parent clonazepam may also result in post-sample collection or postmortem formation of the 7-amino analog. For these reasons, this study seeks to demonstrate that 7-aminoclonazepam should be a requisite analyte included in the analysis of blood samples as a marker for clonazepam use.

A confirmatory Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method for several common benzodiazepines, which included clonazepam and 7-aminoclonazepam, was developed and validated. Samples were prepared by protein precipitation followed by a supported liquid extraction. High-Pressure Liquid Chromatographic (HPLC) separation was performed on a biphenyl column. Positive ion detection was performed by a triple quadrupole MS/MS operating in Multireaction Monitoring (MRM) mode.

More than 6,000 blood samples collected from postmortem and DUID cases across an eight-month time period were analyzed for the presence of benzodiazepines. The samples were first screened by a validated immunoassay process and non-negatives confirmed by the validated LC/MS/MS method at a threshold of 5ng/mL. Confirmed positives for clonazepam and/or 7-aminoclonazepam were evaluated based on concentration, case type (postmortem vs. DUID), drug co-positivity, etc.

Five hundred ninety-seven samples (9.7%) confirmed positive for one or both clonazepam markers. While the majority of the positive samples confirmed for both parent and metabolite/degradant (74.9%), 144 samples (24.1%) were detected above the threshold for 7-aminoclonazepam only. Parent clonazepam with no 7-aminoclonazepam present was detected in only six samples (1.0%). No distinction was obvious between case type for the detection of 7-aminoclonazepam only. The cause of metabolite/degradant-only results could be due to the possibility of differences in time since ingestion, metabolic variations, potential drug-drug interactions, or degradation due to instability; however, regardless of source, the addition of 7-aminoclonazepam as a marker for clonazepam use appears to provide supporting information that could be valuable to the interpretation of the toxicology results.

7-Aminoclonazepam, Postmortem, DUID

K69 A Case of Death by Diclazepam: Lorazepam in Disguise

Fessessework Guale, DVM, Harris County Institute of, Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054; Warren C. Samms, PhD, 1885 Old Spanish Trail, Houston, TX 77054; Jeffrey Walterscheid, PhD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; and Dana L. Johnson, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will be informed regarding the metabolism and toxicity of diclazepam, a potent designer benzodiazepine not approved for medicinal use. In the past few years, new types of benzodiazepines have emerged for recreational use through online portals. These selections often include phenazepam, pyrazolam, etizolam, and diclazepam, among other offerings. Currently, there are no other reports of illicit diclazepam use or cases of fatal toxicity.

This presentation will impact the forensic science community by exploring the first report of a death involving the toxic effects of diclazepam and its associated metabolites.

Diclazepam (2-chlorodiazepam) is a functional analog of diazepam, which has been alleged by recreational users to have a ten-fold higher potency. Diclazepam powder and 1mg or 2mg compressed tablets are sold online as "research chemicals not for human consumption." Based on published data obtained from a human study, diclazepam has an average elimination half-life of 42 hours and metabolizes into several pharmacologically active benzodiazepines, namely delorazepam, lorazepam, and lormetazepam, which can be detected in urine for 6, 19, and 11 days, respectively.

This study reports the death of a healthy 27-year-old man who was discovered unresponsive at home by a friend. The decedent was observed to have a prominent white foam cone coming from his mouth and blood-tinged white foamy fluid coming from his nostrils. Several alcohol bottles, a jar of greenish-brown powder labeled "Mitragayna Speciosa," and a cup of greenish-brown powder-filled capsules were discovered at the scene. No other prescription, over-the-counter medication, or illicit drugs were noted. The decedent reportedly was a strong believer in the use of herbal medicine.

Autopsy findings were significant for pulmonary edema and congestion, white frothy fluid within the airways, brown-yellow granular gastric contents, urinary retention, and cerebral edema. Comprehensive forensic toxicology testing revealed the following: negative blood alcohol screen and a positive Enzyme-Linked Immuno-Sorbent Assay (ELISA) blood screen for benzodiazepines. Quantitation of benzodiazepines by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) revealed 0.62mg/L and 0.44mg/L lorazepam in the blood and stomach contents, respectively. Additional drug screening by Gas Chromatography/Mass Spectrometry (GC/MS) was performed and found the presence of delorazepam and mitragynine in the blood and stomach contents. A breakdown product of lorazepam was also identified in both the blood and stomach contents, which is the result of thermal decomposition of lorazepam in the process of GC. The absence of prescription history for lorazepam and the positive identification of delorazepam in the postmortem samples triggered further investigation to elucidate the parent drug and other possible metabolites. The blood sample screened by Liquid Chromatography/Time-Of-Flight/Mass Spectrometry LC/TOF/MS revealed the presence of the parent drug diclazepam, as well as the active metabolites delorazepam, lorazepam, and lormetazepam in addition to ritalinic acid and mitragynine. The quantitation result of ritalinic acid by LC/MS/MS is 0.11mg/L in the blood. Quantitation of mitragynine was not pursued, because pharmacokinetic data from human study characterizing the toxic and lethal levels of mitragynine is not available. Since mitragynine has opioid activity, its presence with other sedatives is considered as contributory to the effect of sedation. Analysis by the Harris County Institute of Forensic Sciences (HCIFS) Drug Chemistry Laboratory on one of the capsules obtained from the scene revealed the presence of mitragynine, but no trace of diclazepam or any other substance.

Based upon the autopsy findings and toxicology results, the forensic pathologist classified the manner of death as accidental, with the cause considering the contributions of lorazepam and the other upstream benzodiazepine derivatives in combination with mitragynine. This is a prime example of a postmortem toxicology case whereby an extensive analytical workup utilizing sophisticated instrumentation played a paramount role in aiding the pathologist in the determination of the cause and manner of death when scene information yielded few clues about the origins of the toxic substances involved.

Diclazepam, Mitragynine, LC/MS/TOF

K70 Fatal Toxicity Involving 3-Methoxyphencyclidine (3-MeO-PCP)

Amelia Romoser, PhD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; and Jeffrey Walterscheid, PhD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054*

WITHDRAWN

K71 A Crazy Mini Heroin Epidemic in Richmond, Virginia

Carl E. Wolf II, PhD, Virginia Commonwealth University-Health, PO Box 980165, Richmond, VA 23298-0165; Michelle Hieger, DO, Virginia Commonwealth University- Health, PO Box 980401, Richmond, VA 23298-0401; Brandon K. Willis, DO, Virginia Commonwealth University-Health, PO Box 980401, Richmond, VA 23298-0401; and Alphonse Poklis, PhD, Virginia Commonwealth University, Dept of Pathology-Toxicology Laboratory, Box 98-165 MCVH/VCU Station, Richmond, VA 23298-0165*

After attending this presentation, attendees will recognize the signs and symptoms of clenbuterol adulterated heroin and will be aware of the method development and validation of a method for the determination of clenbuterol in biological specimens.

This presentation will impact the forensic science community by presenting the clinical and potentially antemortem signs and symptoms of clenbuterol exposure.

Adulteration of illicit drugs like heroin is not an uncommon occurrence. This occurs to increase profit margin for the dealer, but users are then unaware of these products' purity or composition. Some adulterants may be relatively innocuous while others can result in moderate to severe toxicological reactions. Clenbuterol is a β2-adrenergic agonist, but it acts like an anabolic steroid and causes muscle building. This provides clenbuterol with veterinary uses, but it is not United States Food and Drug Administration approved for human use; it is also a substance banned by the World Anti-Doping Agency and the International Olympic Committee. Clenbuterol has occasionally been reported as a heroin adulterant. This study describes a recent cluster of hospitalized patients with confirmed clenbuterol exposure resulting in serious clinical effects. Ten patients presented to emergency departments in the Richmond area over a ten-day period in the spring with unexpected symptoms shortly after heroin use. Heroin exposure was delivered by the following routes: five patients reported insufflation, three reported intravenous injection, and two patients were unaware.

The objective of this project was to develop a method for the qualification and quantification of clenbuterol in biological specimens.

Specimens were extracted using ISOLUTE® Supported Liquid Extraction (SLE) HCX columns. In brief, serum specimens were analyzed using a seven-point calibration curve ranging from 5ng/ml to 500ng/ml and quality control samples (5ng/ml, 15ng/ml, and 400ng/ml). Urine specimens were analyzed using a nine-point calibration curve ranging from 5ng/ml to 2,500ng/ml and quality control samples (5ng/ml, 15ng/ml, and 2,000 ng/ml). Clenbuterol and clenbuterol-d9 were extracted from the specimen using 0.5M ammonium hydroxide and ethyl acetate. The ethyl acetate was evaporated to dryness using a Biotage® TurboVap® 96 with nitrogen gas at 37°C. Analysis was performed using a Waters® ACQUITY® UPLC® with a TQD mass spectrometer, with positive Electrospray Ionization (ESI). The column was an ultra biphenyl 3µM, 2.1mm x 50 mm. The mobile phase was 10mM ammonium formate in water (A) and methanol (B) with a 95:5 to 5:95 gradient over three minutes.

Seven patients presented to the emergency department with their findings summarized. All patients were male with a median age of 40 years (range 28 years-46 years). Presenting symptoms included chest pain (6/7), dyspnea (5/7), palpitations (5/7), and nausea/vomiting (4/7). Troponin was positive in six patients at some point during their hospitalization. Three patients underwent cardiac catheterization; all revealed no significant coronary artery disease. Qualitative and quantitative clenbuterol concentrations were detected in the serum and urine of all seven patients. The results are as follows: serum median concentration 15ng/ml (range 6-38), urine median concentration 1,367ng/ml (range 13-3,389). The observed r^2 values for the calibration curves were 0.99 or better. The limit of quantitation was administratively set at 5ng/ml for both serum and urine specimens. Validation criteria for calibrators and quality control specimens as well as carryover, matrix effect, precision, process efficiency, recovery, and specificity were acceptable.

The presence of drug adulterants in illicit drugs may result in atypical presentations of intoxication. Presentation of adrenergic symptoms and/or chest pain with hypokalemia, lactic acidosis, and hyperglycemia in heroin use suggests heroin adulteration with a beta agonist drug like clenbuterol. Clenbuterol adulteration of heroin can result in serious signs and symptoms and often requires hospitalization. A method is presented for the qualification and quantification of the clenbuterol in biological specimens.

Clenbutreol, Heroin, LC/MS/MS

K72 Rise in Fentanyl Derivatives Acetyl and Butyryl Fentanyl Detection in Blood and Serum Coinciding With Rise in Opiate and Novel Psychoactive Substances (NPS) Use

David Buzby, BS, 3102 Georgetown Road, Cinnaminson, NJ 08077; Donna M. Papsun, MS, Willow Grove, PA 19030; Daniel S. Isenschmid, PhD, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090; and Barry K. Logan, PhD, NMS Labs/CFSRE, 3701 Welsh Road, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to describe the emerging group of fentanyl analogs, the development of an analytical assay utilizing Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) for their detection in biological fluids, and be able to discuss concentrations of the drugs determined in blood from toxicology casework.

This presentation will impact the forensic science community by highlighting the new trend of fentanyl analogs that are being detected with increasing frequency in forensic casework.

With the rise in opiate use in the United States, it is not unexpected that there would be a corresponding rise in related drugs either contaminating or counterfeiting the heroin supply. Fentanyl is commonly seen as either a contaminant or a replacement product in street heroin; however, in recent times, certain additional fentanyl derivatives are garnering their own market share and appearing in forensic casework. Two of the fentanyl derivatives that have been detected in chemical and toxicological analyses are acetyl fentanyl and butyryl fentanyl. Reports of acetyl fentanyl began in 2013, with overdoses being reported in Rhode Island, Pennsylvania, and Louisiana. Butyryl fentanyl, a homologue of fentanyl, started to be identified in forensic casework in late 2014. Due to the quick proliferation of cases that were being linked to fentanyl derivatives, as well as other designer opioids, there was a need to develop an assay for the detection of these compounds in forensic toxicology specimens.

A quantitative procedure was developed for the identification and quantitation of four analytes (acetyl fentanyl, butyryl fentanyl, and two other designer opioids not related to fentanyl, MT-45 and AH-7921) using positive mode Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). Extraction of these compounds was achieved using acetonitrile for protein precipitation, followed by adding phosphate buffer to the transferred supernatant, with a final step of solid phase extraction using cation exchange columns. Analytical separation was achieved using a silica-based, 2.1mm x 50mm, 1.8 micron column, using mobile phases of ammonium formate at pH4.0 and acetonitrile. Internal standards consisted of $^{13}C_6$-acetyl fentanyl and dueterated AH-7921.

The calibration curve was comprised of six calibrators, at 0.1ng/mL, 0.2ng/mL, 0.5ng/mL, 2ng/mL, 5ng/mL, and 10ng/mL. Calibration experiments (*n*=5) demonstrated acceptable performance, with correlation coefficients >0.999 for all four analytes. Between-run precision, total precision, and accuracy experiments for acetyl and butyryl fentanyl (*n*=15) demonstrated acceptable performance (< +/- 8.7%). The limit of detection was determined to be 0.003ng/mL in both blood and serum for acetyl and butyryl fentanyl. Both fentanyl derivatives are stable in blood in all tested conditions for up to 30 days; acetyl fentanyl and butyryl fentanyl are stable for up to 14 days in serum at room temperature, but 30 days at refrigerated and frozen conditions.

Of the designer opioids included in this panel, positives have been reported for both acetyl fentanyl and butyryl fentanyl. From July 2013 to June 2015, 183 cases of acetyl fentanyl use have been detected by the laboratory. Blood concentrations range from 0.11ng/mL to 3,800ng/mL, with average and median concentrations falling at 150ng/mL and 30ng/mL, respectively. Butyryl fentanyl has been detected in only two cases thus far, but testing for this drug was not implemented until May 2015. Butyryl fentanyl was found at 20ng/mL in one case and in a second case was detected at 3ng/mL, in addition to 0.65 ng/mL of acetyl fentanyl.

In 81 (44%) cases positive for acetyl fentanyl, fentanyl and/or norfentanyl were also detected. In 52 (64%) of these positive cases, both acetyl fentanyl and fentanyl were greater than 5ng/mL. In 15 (18%) cases, fentanyl was greater than 5ng/mL, accompanied by smaller amounts of acetyl fentanyl.

With the recent federal scheduling of acetyl fentanyl, it is unknown if that will deter future manufacturing, distribution, and use of this drug or if other analogs will replace its presence. Forensic toxicology laboratories as well as other members of the drug monitoring community must be cognizant of the expansion of fentanyl derivatives and the other designer opioids whose prevalence is increasing in forensic casework, and consider their analysis in cases where circumstances of death cannot be explained by traditional opioid drugs.

Acetyl Fentanyl, Butyryl Fentanyl, Opioid

K73 Fatal Methadone Intoxication in an Infant Listed as a Homicide

Alessandro Bonsignore, MD, PhD, University of Genova, Department of Legal & Forensic Medicine, Via de Toni 12, Genova, Liguria 16132, ITALY; Francesco Ventura, MD, PhD, Department of Legal Medicine University of Genova, via de Toni, 12, Genova 16132, ITALY; and Cristian Palmiere, MD, Curml Centre Universitaire, Romand Medicine Legale, 4 chemin de la Vulliette, Lausanne 25, VD 1000, SWITZERLAND*

After attending this presentation, attendees will better understand fatal and non-fatal accidental methadone overdoses in the children of drug-dependent parents as well as infrequent cases of fatal methadone intoxications in children due to deliberate drug administration by adults seeking to sedate and calm them. A review of the literature on this topic is also provided.

This presentation will impact the forensic science community by highlighting the importance of considering all potentially relevant toxicological data in order to formulate appropriate hypotheses concerning the cause and manner of death. In addition, this presentation will impact the forensic science community by emphasizing the usefulness of hair analysis to identify threatening situations for the children of drug-dependent parents and possibly support measures by the authorities to recognize and intervene in these potentially fatal situations.

Methadone is a synthetic, long-lasting opioid whose structure has no relation to morphine or other opium alkaloids. It is a long-acting μ-receptor agonist with pharmacologic properties qualitatively similar to those of morphine; however, it is characterized by a longer half-life elimination and improved oral bioavailability. The drug has a long, successful history of moderate-to-severe pain relief in opiate-dependence substitution treatment. Recreationally, it is used for its sedative and analgesic effects.[1]

Fatal and non-fatal accidental methadone overdoses in the children of drug-dependent parents have been previously reported by numerous authors. In most of these cases, intoxication is an unfortunate consequence of methadone availability at home, when one of the parents or relatives are on a methadone maintenance program, and may be related to inappropriate methadone storage or the liquid preparations resemblance to common soft drinks. Voluntary administration with the purpose of calming or sedating a child that eventually results in the infant's death is extremely infrequent, though it has occurred.1-7

In this presentation, an autopsy case pertaining to a 32-month-old infant who was repeatedly exposed to methadone by his parents will be described. Autopsy revealed a coartaction of the aorta with a focal stenosis located at the junction of the distal aortic arch and the descending aorta. Left ventricular hypertrophy was also observed. Both of these findings were considered to have not played a role in the child's death. Methadone was detected in the femoral blood (0.633mg/l), urine (5.25mg/l), bile (2.64mg/l), and gastric contents (1.08mg). A segmental hair analysis was performed by Gas Chromatography/Mass Spectrometry (GC/MS) and showed the presence of methadone in both the proximal (3.11ng/mg) and distal (4.91ng/mg) portion of the hair. Methadone was also detected in nail samples. A segmental hair analysis (performed on the younger brother of the deceased) revealed the presence of methadone in both the proximal (0.40ng/mg) and distal (0.93ng/mg) segments, as well as the presence of 6-monoacetylmorphine exclusively in the distal portion.

In this case, it was concluded that the deceased child had deliberately received repeated methadone administered by one (or both) of the parents, most likely in order to induce sedation. The cause of death was determined to be methadone intoxication and the manner of death was listed as homicide. Hair testing revealed continual methadone exposure in both the deceased and his younger brother. Hair analysis also demonstrated that the latter was exposed to heroin.

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Methadone, Overdose, Infant

K74 Case Report: Two Child Fatalities Due to Heroin/Fentanyl Exposure

Rebecca T. DeRienz, MS, 6034 Jamesport Drive, Westerville, OH 43081; Daniel Baker*, 520 King Avenue, Columbus, OH 43201; Rachel M. Barnett, BCJ, 1949 Rea Avenue, Columbus, OH 43223; Jennifer M. Hogue, MS, 22250 Reed Road, Marysville, OH 43040; Nancy E. Kelly, 520 King Avenue, Columbus, OH 43201; John A. Daniels, MD, 520 King Avenue, Columbus, OH 43201; and Anahi Ortiz, Franklin County Coroner Office, 520 King Avenue, Columbus, OH 43201*

After attending this presentation, attendees will better understand the successful toxicological investigation of two unrelated cases involving children that suffered unexpected lethal heroin/fentanyl exposures.

This presentation will impact the forensic science community by sharing postmortem findings of two cases, thereby advantageously making additional reference information available. This presentation will also communicate the need for the forensic toxicologists to routinely screen for fentanyl regardless of initial case circumstances and further investigate depressed immunoassay results in light of other analytical findings.

Drug user environments have the potential to inadvertently affect innocent bystanders through all possible routes of exposure. Lethal intoxications of children in these environments do not appear to occur often and, therefore, published knowledge of these cases is sparse.

This case report presents two postmortem cases of unrelated children found to have heroin and fentanyl in their systems. Although heroin and fentanyl separately and in combination across the country have been a well-documented nationwide problem, the cases presented here represent the youngest heroin/fentanyl intoxications published to date. Both cases were not originally investigated as suspicious and were anticipated to be natural or accidental in nature. The first case involves an 11-month-old male found unresponsive at his residence with no suspicion of foul play. The second case concerns a 14-month-old female initially reported to be found unresponsive in her car seat after eating soft candies. Upon scene response, Case 1 appeared to be an unsafe sleeping situation or potential Sudden Unexplained Infant Death (SUID) while Case 2 seemed to be an accidental choking on food. Toxicology screening was performed by **Enzyme-Linked Immuno-Sorbent Assay** (ELISA) in whole blood, demonstrating a presumptive positive fentanyl result for Case 1 and a presumptive positive opiate and fentanyl result for Case 2. Urine, if available, was screened by Gas Chromatography/Mass Spectrometry (GC/MS) in full scan mode after Solid Phase Extraction (SPE). Quantitative opiate confirmation of 6-monoacetylmorphine (6-MAM) and morphine was achieved in blood and gastric contents for each case using SPE and GC/MS in selected ion monitoring mode. Additionally, diacetylmorphine (heroin) was analyzed qualitatively in gastric contents by GC/MS in full scan mode. Quantitative analysis of fentanyl and norfentanyl in blood and gastric contents was achieved by SPE and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) equipped with an Electrospray Ionization (ESI) source and operated in positive ionization mode. Analytical findings are summarized in Table 1.

After the toxicology results were reported, follow-up investigation with witnesses by the local police department revealed heroin use in the home related to Case 1. The high levels of fentanyl and 6-MAM along with the presence of diacetylmorphine in the gastric of Case 1 suggests the child consumed heroin and fentanyl orally. The police investigation from Case 2 revealed that the child was taken inside a known drug house prior to becoming unresponsive. The circumstances surrounding the second child's exposure to heroin are undetermined at this time.

Table 1: Toxicology Results

			Source		
	ELISA Screen Results in Whole Blood	**Compound of Interest**	**Heart Blood Result (ng/mL)**	**Gastric (ng/mL) *(Total Recovered = ~100mL)***	**Urine Result**
CASE#1	FENANYL (POS) *33% b/b0*	Fentanyl	14	1143	POS
		Norfentanyl	1.6	Not Detected	POS
	OPIATES (ND) (Depressed Result) *34% b/b0*	Diacetylmorphine	Not Detected	POS	Not Detected
		6MAM	Not Detected	523	Not Detected
		Morphine	Trace *(between 5-20)*	50	POS

	ELISA Screen Results in Whole Blood	Compound of Interest	Thoracic Blood (ng/mL)	Gastric (ng/mL) *(Total Recovered = ~2mL)*
CASE #2	FENANYL (POS) *17% b/b0*	Fentanyl	20	101
		Norfentanyl	Not Detected	Not Detected
	OPIATES (POS) *6.5% b/b0*	Diacetylmorphine	Not Detected	Not Detected
		6MAM	Trace *(between 2.5-10)*	163
		Morphine	106	86

Heroin, Fentanyl, Pediatric

K75 Postmortem Pediatric Forensic Toxicology

Robert A. Middleberg, PhD, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090; Nikolas P. Lemos, PhD, OCME, Forensic Lab Division, Hall of Justice, N Terrace, 850 Bryant Street, San Francisco, CA 94103; Tracey S. Corey, MD, OCME, 810 Barret Avenue, 7th Fl, Louisville, KY 40204; Alane Olson, MD*, Clark County Coroner's Office, 1704 Pinto Lane, Las Vegas, NV 89106; Karen Cline-Parhamovich, DO*, University of New Mexico, Office of the Medical Investigator, 1101 Camino del Salud, Albuquerque, NM 87016; Kenneth E. Ferslew, PhD*, East Tennessee State University, Section of Toxicology, Box 70422, Johnson City, TN 37614; and Robert Kronstrand, PhD*, National Board of Forensic Medicine, Dept of Forensic Toxicology, Artillerigatan 12, Linkoping SE 587 58, SWEDEN*

After attending this presentation, attendees will better appreciate the challenges unique to toxicological findings in postmortem pediatric cases. Attendees will learn interpretive guidelines for pediatric cases involving forensic toxicology in both a general and case-specific sense.

This presentation will impact the forensic science community by further delineating the interpretive aspects of toxicological findings in the pediatric population.

In this 17th Annual Special Session within the Toxicology section, pediatric cases involving toxicological findings are discussed. As a relative dearth of interpretive information involving toxicological findings in the pediatric population exists, this session is a forum to help elucidate and clarify such issues. The format is a short case presentation, including pharmaco-toxicokinetic data and other relevant ancillary information followed by audience participation to provide interpretive clarity around the case-specific impact of the toxicological findings. This session, attended by various sections of the American Academy of Forensic Sciences, allows for diverse perspectives of case issues that lead to integrative consensus, or differing opinions, as to cause of death in children.

Four cases will be presented that highlight the difficulty in assessing the role of toxicants in each case. Tracey Corey, MD, Alane Olson, MD, and Robert Kronstrand, PhD, will be reviewing cases from their years of experience as forensic pathologists and toxicologist, respectively, that highlight the issues and confounders in the pediatric population.

Ken Ferslew, PhD, and Karen Cline-Parhamovich, DO, will be discussing a case tentatively titled "Pediatric Soda Death, Complicated by Potential Furosemide Ingestion." This case will highlight the less concept established by Paracelsus in the 1500s that the dose makes the poison, especially when it comes to substances generally considered benign.

The case studies presented reflect current-day findings in medicolegal investigations of childhood deaths. In years past, discussions of these types of cases have been educational and demonstrative of the issues in this special population. Only through these continued case studies and audience participation can there be shared perspectives on the meaning of the toxicological findings.

Pediatric, Postmortem, Toxicology

LAST WORD SOCIETY

LW1 The Best Forensic Scientist You've Never Heard of: Wilmer Souder and the Early History of Forensic Science at the National Bureau of Standards (NBS)

Kristen Frederick-Frost, PhD, NIST, 100 Bureau Drive, MS 2500, Gaithersburg, MD 20899; Robert M. Thompson, BS, NIST, Special Programs Office-Forensic Sciences, 100 Bureau Drive, MS 8102, Gaithersburg, MD 20899; and John M. Butler, PhD*, NIST, 100 Bureau Drive, MS 4701, Gaithersburg, MD 20899*

After attending this presentation, attendees will have a new appreciation for the early history of forensic science in the United States and the role that the National Bureau of Standards (now the National Institute of Standards and Technology (NIST) played in forensic casework from the 1920s to the 1950s.

This presentation will impact the forensic science community by providing a new appreciation for the early activities of the NBS in the formation of the Federal Bureau of Investigation (FBI) Laboratory and other early federal forensic science activities.

Rum-running, murder, kidnapping, and forgery are not subjects of study that are typically associated with the NBS; however, they were the bailiwick of NBS physicist Wilmer Souder (1884-1974) and the crime laboratory which he developed. Until recently, the early history of forensic science at the NBS has not been explored. Interest in this topic has increased as the visibility of NIST's current research and efforts in forensic science rises; specifically, its involvement with the National Commission on Forensic Science, the Organization of Scientific Area Committees, and the newly formed NIST Center of Excellence for Forensic Science. Reconstructing Souder's career and impact on forensic science between 1920 and 1950 provides insights into the development of the discipline's methodologies and its path to professionalization — issues that are still of interest today.

Skimming the literature immediately available on Wilmer Souder paints a portrait of a rather remarkable physicist, not a criminalist. He first came to the NBS in 1911 after completing a master's degree in physics from Indiana University. In 1913, he left to pursue a PhD in physics at the University of Chicago. Souder's dissertation concentrated on the photoelectric effect, a topic which would help earn his advisor, Robert Millikan, the 1923 Nobel Prize in Physics. By 1917, Souder returned to the NBS and oversaw research regarding length measurement and the thermal expansion of materials. As such, he was an ideal candidate for the Army to contact in 1919 to research improvements in dental fillings. Many publications and suggested improvements for dental amalgams followed, earning Souder recognition as an authority in the field. To this day, the International Association for Dental Research has an award named in his honor.

But this was just one side to Souder's research. Hints of his involvement in forensic science are found tucked in between the dental research articles and related awards in his file in the NIST archives. Newspaper clippings offered the first real insight into his research for several high-profile criminal cases, most notably Souder's contribution to the handwriting analysis for the Lindbergh baby kidnapping case. A January 1954 *Washington Post* article called him "one of the Nation's best but almost certainly one of its least-known criminologists." Another newspaper article headline announced his retirement from the NBS with the headline "The Underworld Will Approve." It was clear there was more to Souder than met the eye.

The recent rediscovery of nine Souder notebooks dating between 1929 and 1953 revealed that his impact on forensic science extended far beyond a handful of high-profile cases — by several orders of magnitude. From handwriting analysis to ballistics identification, Souder interacted with a host of federal agencies and investigators. He conducted numerous analyses, issued reports, and would often back up his findings by providing expert testimony in court. The notebooks allow us to trace these interactions as well as the various networks between early forensic scientists and laboratories. Souder didn't just build a crime laboratory at the NBS; he was active in training the next generation of practitioners. His involvement in the development of the FBI laboratory is a notable example.

Souder imparted his views on how to both draw conclusions and convince others of their validity. He strongly advocated for processes, methodologies, and controls that would elevate the profession as a whole. While he may have worked quietly behind the scenes, Souder's impact was wide-reaching.

Forensic History, NIST, Handwriting Analysis

LW2 Giving Voice to a Serial Killer: Clinical Implications

Katherine Ramsland, PhD, DeSales University, 2755 Station Avenue, Center Valley, PA 18034*

After attending this presentation, attendees will learn how the use of a guided autobiography, which identifies developmental factors in extreme offenders, helped to structure a killer's self-report and ultimately benefited criminology and law enforcement.

This presentation will impact the forensic science community by illustrating to the forensic community a layered approach to extreme offenders that affirms overlooked methods for identifying subtle but important dimensions of behavior.

Since the 19th century, mental health professionals have sorted through multiple factors that seem to set an individual on a violent path. Around 1830, these specialists began to collectively systematize their knowledge. Later that century, French pathologist Alexandre Lacassagne urged offenders to ponder their lives and acts in writing.[1] He instigated "criminal autobiographies," hoping to identify common and unique developmental factors. Although self-report has its limitations, it can also be data-rich, especially when coupled with observation. Lacassagne used it as a tool for greater comprehension.[2]

In 1930, Karl Berg interviewed serial killer Peter Kürten before his execution. Kürten confessed freely, which became the basis for Berg's now-classic book, *Der Sadist*.[3] Berg's guided interviews and analytical observations became a model for other professionals.

Lacassagne and Berg inspired an approach to the "Bind, Torture, Kill (B.T.K.)" serial killer, Dennis Rader, for specific types of questions to guide his life story. The goal was to provide insight for law enforcement, criminologists, and psychologists. Although Rader gave a lengthy confession to the police, they were interested primarily in the facts of each incident. They did not seek to understand or contextualize him with what is known about such offenders. They also paid little attention to his confession behavior.

There's a clear difference between the facts of a case and the way killers tell their story. Lacassagne and Berg both noted this. They retrieved important information about motives, pre- and post-crime behavior, fantasies, compartmentalized personalities, and the role of mental deviance and disorder.

However, an insight about self-reflection described by Danish philosopher Søren Kierkegaard suggests a different kind of "data-mining" that few criminologists are trained to exploit. In *Concluding Unscientific Postscript*, Kierkegaard described the difference between the *what* and the *how*, as well as how the latter shapes the former: "An existing individual is constantly in process of becoming; the actual existing subjective thinker constantly reproduces this existential situation in his thoughts, and translates all his thinking into terms of process."[4] Today, the clinical and research communities identify this as cognitive bias. Always present, it sheds as much light on individuals' traits and behaviors as does what they state in their self-narratives.5

Even those offenders who make an honest effort to study themselves, Kierkegaard would say, cannot fully grasp all aspects of their experience. Their "pre-reflective engagement" (the "how") is infused with their idiosyncratic manner of experiencing. They can describe certain things but will inevitably have blind spots. Within these blind spots are revelatory aspects of personality.

Rader's narrative was partially structured with the psychological methods that Lacassagne, Berg, and Kierkegaard laid out. He was quite expressive, but his use of language, aimed to control his world, blocked him from noticing his own behaviors and attitudes that were nevertheless apparent to an observer. Several are described in this presentation.

In conclusion, there is more to an offender's story than what he might say, no matter how verbal. To give Rader his voice for a book about his life, earlier work has set a precedent for the type of observation and questioning used. This approach will benefit law enforcement, criminology, and psychology.

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Forensic Interview, Serial Killer, Criminal Autobiography

LW3 Capital Punishment by Lethal Injection

David M. Benjamin, PhD, 77 Florence Street, Ste 107N, Chestnut Hill, MA 02467-1918*

The goals of this presentation are to: (1) review the history of lethal injection as a form of capital punishment; (2) examine the original drugs used for this purpose; (3) identify subsequent changes in the drugs and the deficiencies they posed to the process; and, (4) summarize problems encountered with lethal injection and arguments against lethal injection posed by opponents.

This presentation will impact the forensic science community by addressing the question: Is the current method of lethal injection the best we can do?

Executions were first carried out with the guillotine, the "firing squad," and finally the electric chair and the gas chamber; however, on May 11, 1977, Oklahoma's state medical examiner, Jay Chapman, MD, proposed a new method of execution, in which an ultra-short-acting barbiturate in combination with a chemical paralytic were administered via intravenous injection. "Chapman's Protocol" was introduced into the Oklahoma legislature and was quickly adopted.

On August 29, 1977, Texas adopted the new method, switching to lethal injection from electrocution. On December 7, 1982, Texas became the first state to use lethal injection to carry out the execution of Charles Brooks, Jr. From 1977 to 2004, 37 of the 38 states employing capital punishment introduced lethal injection statutes.

The original protocol utilized sodium pentothal, a rapidly-acting "induction agent" to induce unconsciousness. This was followed by injection of pancuronium bromide, a curare-like agent that paralyzed the respiratory muscles and diaphragm. Lastly, potassium chloride, a "cardioplegic" drug used to stop the heart from beating during open-heart surgery, was injected to poison the cardiac electrical conduction system and stop the heart. On October 15, 2013, Florida was the first state to switch to midazolam, the first drug in a new three-drug protocol. On November 14, 2013, Ohio followed. The Ohio protocol, developed after the incomplete execution of Romell Broom, tried to ensure the rapid and painless onset of anesthesia by using only sodium thiopental and eliminating the use of pancuronium bromide and potassium as the second and third drugs. It also provided for a secondary fail-safe measure using intramuscular injection of midazolam (a water-soluble form of diazepam) and hydromorphone in the event intravenous administration of the sodium thiopental proved problematic

After sodium thiopental began being used in executions, Hospira, Inc., the only American company that made the drug, stopped manufacturing it due to its use in executions. The subsequent nationwide shortage of sodium thiopental led states to seek other drugs. On December 16, 2010, pentobarbital, a drug often used for animal euthanasia, was used as part of a three-drug "cocktail" for the first time during the execution of John David Duty in Oklahoma. On March 10, 2011, pentobarbital was used as a single drug in lethal injection when Johnnie Baston was executed in Ohio.

The American Medical Association argued that a doctor "should not be a participant" in executions in any professional capacity with the exception of "certifying death, provided that the condemned has been declared dead by another person" and "relieving the acute suffering of a condemned person while awaiting execution." Due to physician resistance, some states passed laws stating that participation in a lethal injection is not to be considered practicing medicine. Delaware's law read, "the administration of the required lethal substance or substances required by this section shall not be construed to be the practice of medicine…." Still, many physicians declined to participate in lethal injections, requiring poorly trained technicians to calculate doses and do the actual intravenous injections.

On April 29, 2014, Clayton Lockett died of a heart attack during a failed execution attempt at the Oklahoma State Penitentiary in McAlester, OK. Technicians could not locate a good venous access due to his prior days of dehydration and the technical inadequacies of the staff. He was administered a mixture of drugs that had not previously been used for executions in the United States and survived for 43 minutes before being pronounced dead. Lockett convulsed and spoke during the process and attempted to rise from the execution table 14 minutes into the procedure, despite having been declared unconscious. Was this technique an improvement over prior methods of lethal injection, or did it subject the recipient to more pain and suffering? Ethical and pharmacologic issues will be discussed.

Capital Punishment, Lethal Injection, Flawed Methods

LW4 The Short Life and Death of George Junious Stinney, Jr.: A Cold Case Review Illustrating Difficulties in Revisiting the Science and the Law in a Long-Ago Case

Peter J. Stephens, MD, 100 Club Drive, Ste 135, Burnsville, NC 28714*

WITHDRAWN

LW5 Small Town Forensics in the Land of Oz

Bryan R. Burnett, MS, Meixa Tech, PO Box 844, Cardiff, CA 92007-0844*

After attending this presentation, attendees will understand that criminal justice in small jurisdictions in the United States can be far from just.

This presentation will impact the forensic science community by illustrating how small-town forensics are often marred by evidence mishandling, prosecution-biased defense attorneys, and junk science.

Case 1: Racism by the defense council in a death penalty case is presented. The defense attorney was witnessed saying he hoped his client would be convicted. In the same case, there was a failure to maintain the integrity of evidence after trial (evidence bags had been ripped open while in court storage). In one of those items, the shirt of the victim had been laundered, making it impossible in a reanalysis of the shooting to determine muzzle-target distances of three victim bullet strikes. A shooting reconstruction was presented in court by a police officer who had no apparent training in crime scene processing and analysis. Photographs of alleged bullet strikes were presented in court but showed no evidence of bullet strikes. (*People of the State of California vs. Clifton Perry*, Hanford, CA, Case No. L955500, 1995). The defendant is on death row.

Case 2: A well-known crime scene reconstruction expert affirmed his shooting hypothesis of suicide by intraoral shotgun discharge using wooden boxes to simulate the head of the victim. The plywood boxes were fabricated with holes cut in them for the nose and mouth and bags of colored water inside were used to simulate blood. Polyvinyl Chloride (PVC) tubes were inserted into the bottom of the boxes to simulate trachea. The victim actually died by homicide which was determined by the preponderance of crime scene and autopsy evidence. (United States Congress Armed Services Authorization Act of 2004, Section 584; Federal Contract #HQ0095-04-C-0022, 2004). The victim's death is still officially listed as suicide.

Case 3: A criminalist reconstructed a crime scene in which he ignored or did not recognize the significance of physical evidence; the evidence of multiple assailants in the homicide was disregarded to support a one-assailant hypothesis. A medical examiner amended his original autopsy report to support the reconstruction after a meeting of the prosecutor and his experts prior to trial. The defense attorney refused to present exculpatory evidence to the jury. (*People of the State of California vs. Corey Lyons*, Santa Barbara, CA, Case No. 1296247, 2011). The defendant is serving a life term in prison.

These examples of junk science and unethical behavior are a few of the more outrageous cases encountered by this research. The lack of an outside, anonymous, peer review of prosecution criminalist work product presented in trial is a largely unrecognized problem in the criminal justice system. Despite independent presentation of exculpatory evidence after trial which debunks inculpatory evidence presented in these and other cases, the incarcerated have little chance of official review of new or correctly interpreted evidence unless that evidence is DNA.

Junk Science, Homicides, Small Town Justice

LW6 Houdini on the Crime Scene: Debunking Psychic Sleuthing

Matteo Borrini, PhD, Liverpool John Moores University, RCEAP-School of Natural Science & Psych, Byrom Street, Liverpool L3 3AF, UNITED KINGDOM*

The goal of this presentation is to illustrate the truth behind the claims of alleged psychics regarding the involvement of paranormal abilities in forensic investigations.

This presentation will impact the forensic science community by providing correct information about the supposed involvement of psychics in forensic cases, thereby protecting the reputation of law enforcement.

When media devote attention to a missing person case or a murder investigation that appears to be particularly complex, it is common to have so-called "psychic detectives" offering their help to the police. Various unproven abilities are proposed as tools to locate the missing individual, the victim's body, or the perpetrator. Frequently, mediums declare their paranormal feelings to newspapers or are invited to appear on television shows, and they support their alleged professionalism by claiming to have been previously successfully involved in forensic investigations.

An accurate and scientific analysis of their statements expose a different reality behind the psychic detectives' words.

In the United States, a very popular medium claimed to be a psychic sleuth with an accuracy rate between 87% and 90%. This assertion was examined by skeptical scientists and journalists with the results showing a discrepancy: the confirmable accuracy rate was 0%. The only cases in which this medium was not proven wrong were those that remained unsolved.

Another way to debunk psychic sleuthing, following the teachings of the great magician Harry Houdini, is to demonstrate how illusionists can replicate the same apparently paranormal abilities claimed by mediums by using "magic" methodology. The difference is the goal for which the trick is performed: for fraudulent purposes or for entertainment. Despite this, in the United States mediums are offering courses and classes on how to become a psychic detective, communicate with murder victims, and find their bodies.

One of the most intriguing cases allowing an in-depth understanding of the procedures behind the allegedly successful divination of paranormal investigators is that of the Italian "Lake Soothsayer." The body of a missing young female was found in Lake Como, Lombardia, by apparently following the paranormal indication of a psychic. Since that time, journalists have referred to her as a positive example of psychic sleuthing officially involved with the judicial authority; however, an investigation carried out by the Italian skeptic society Committee for the Investigation of Claims of the Pseudosciences (CICAP) pointed out that the psychic was never appointed or involved with any police force. Most importantly, it was demonstrated that the search during which the corpse was discovered was not conducted following any paranormal clues or advice.

The missing individual had disappeared during a stormy night while driving home; the route she had traveled between the last location she was seen and her house ran along the lake bank. The investigators conducted scuba searches along the shore; however, one area had been left unchecked due to the fact that the bottom of the lake had a steep gradient. In addition, the road above that location had a very dangerous curve before a tunnel entrance and during the night of disappearance, debris from a construction site had been abandoned near it. All of these details were published by media and were very well known when the psychic visited the site two years after the disappearance with a volunteer scuba rescue team, who had previously offered the use of their robotic submarine unit to check the area that had not yet been explored. The clairvoyant claimed to feel the presence of the body from the lake just as the new in-depth survey was planned. When the search discovered the victim in her sunken car in the area not reached by the previous activities, the newspapers stressed the apparent paranormal success.

This case is helpful in clarifying how the psychic achieved the alleged success, by using common sense and the information available from the news, but also how she was never appointed by the forensic investigator. Moreover, the analysis of this event is emblematic in understanding the "myth-making process" by which mass media create "successful" psychic detectives. This is achieved by concealing the non-paranormal methods used, not questioning the assertions regarding the official appointments, and not reporting the unsuccessful performances. The "Lake Soothsayer" herself has tried to make claims concerning other cases, both forensic and historical, but all the locations and clues provided were incorrect. Unfortunately, only skeptic societies provide information regarding these failures, which are not reported by media.

With the support of both the investigative approach and an illusionist background, this presentation exposes several psychic detective cases to demonstrate not only how their stories are unreliable and without any scientific foundation, but also clarifies that law enforcement agencies never ask for help from self-styled clairvoyants or bizarre unscientific consultants.

Psychic Detective, Skeptic, Paranormal Investigation

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Discloses no financial relationships with commercial entities.

Katelyn L. Bolhofner, MA - A53
Maricopa County Office of the Medical Examiner (Other Financial/Material Support).

Christopher R. Bommarito, MS - B172
Discloses no financial relationships with commercial entities.

Claudia M. Bonilla - E76
Discloses no financial relationships with commercial entities.

Eliud Bonilla, BS - C11
Discloses no financial relationships with commercial entities.

Joseph P. Bono, MA - F3
Discloses no financial relationships with commercial entities.

Alessandro Bonsignore, MD, PhD - K73
Discloses no financial relationships with commercial entities.

Julian C.W. Boon, PhD - I18
Discloses no financial relationships with commercial entities.

Matteo Borrini, PhD
Liverpool John Moores University (Employee). - A55
Discloses no financial relationships with commercial entities. - E85, F1, I31, LW6

Michael J. Bosse, MFS - E90
Discloses no financial relationships with commercial entities.

Cory Bosworth, BS - H82
University of Tennessee Health Science Center (Employee).

Sabra R. Botch-Jones, MS, MA - W16
Discloses no financial relationships with commercial entities.

Gregory Bowen - C3
The University of Alabama College of Arts and Sciences Undergraduate Creativity and Research Academy (Grant Support).

Charles Michael Bowers, DDS, JD - G25
Discloses no financial relationships with commercial entities.

Cliff Boyd, PhD - A25
Discloses no financial relationships with commercial entities.

Donna C. Boyd, PhD - A77
Keyence Corporation (Discussion of Commercial Products or Services).

Sean Brady - B32
National Institute of Justice, Office of Justice Programs, U.S. Department of Justice (Grant Support).

Thomas V. Brady, DMD - G24
Discloses no financial relationships with commercial entities.

Stephanie R. Breitenbach, BS - B20
Waters Corporation (Discussion of Commercial Products or

Services).
National Institute of Justice (Grant Support).

Charles H. Brenner, PhD - B97, B174
Brenner, C.H. (Discussion of Commercial Products or Services).

Thomas A. Brettell, PhD
Agilent Technologies, PerkinElmer, Inc (Discussion of Commercial Products or Services). - B72
EMD Millipore, SCIEX, Shimadzu Corporation, Restek Corporation (Discussion of Commercial Products or Services). - B153
Cedar Crest College (Employee). - B72, B153

Alice Briones, DO - W23
DoD DNA Registry a division of the Armed Forces Medical Examiner System (Speakers Bureau).

Kristen A. Broehl, BA - A57
Discloses no financial relationships with commercial entities.

Ryan P. Brokaw, MFS - W7
U.S. Army CID (Employee).

Cristina Enrica Brondoni, MS - E71
Discloses no financial relationships with commercial entities.

Jason W. Brooks, VMD, PhD - H2
Discloses no financial relationships with commercial entities.

Helmut G. Brosz, BASc, PEng - S2
Discloses no financial relationships with commercial entities.

Samuel I. Brothers, BBA - W15
U.S. Customs & Border Protection (Employee).

Anastasia M. Brown, BS - B71
National Institute of Justice, Sam Houston State University (Grant Support).

Catherine O. Brown, BA - B11
Agilent Technologies (Discussion of Commercial Products or Services).
Arcadia University (Other Financial/Material Support).

Katherine M. Brown, PhD - E53
Discloses no financial relationships with commercial entities.

Kimberly Brown, MD - I8
Discloses no financial relationships with commercial entities.

Lyndsey T. Brown, BS - B64
Discloses no financial relationships with commercial entities.

Michael A. Brown, PhD - A122
National Institute of Justice (Grant Support).

Richard S. Brown, MS - D15
MVA Scientific Consultants (Employee).

Theodore T. Brown, MD - H83
Discloses no financial relationships with commercial entities.

Whitney Brown, BS - K18
Arizona Criminal Justice Committee for the National Institute of Justice (Grant Support).

Ann M. Bruhn, MS - G44
Aribex, Inc, DENTSPLY International, Patterson Companies, Inc (Discussion of Commercial Products or Services).

Joshua L. Brunty, MS - C9
Discloses no financial relationships with commercial entities.

Erick P. Bryant, MFS - E19
Discloses no financial relationships with commercial entities.

Clinton D. Buchanan, PhD - B135
Applied Biosystems, Inc, QIAGEN, Inc (Discussion of Commercial Products or Services).
Defense Forensic Science Center, U.S. Army CIL (Employee).

Helio Buchmuller, PhD - B210
Discloses no financial relationships with commercial entities.

Rebecca E. Bucht, PhD - W9
Discloses no financial relationships with commercial entities.

Kristi Bugajski, PhD - H113
IBM Corporation (Discussion of Commercial Products or Services).
Pierce Cedar Creek Institute, Valparaiso University (Grant Support).

Zachary M. Burcham, BS - H4
National Institute of Justice (Grant Support).

Christiana Burgess, BS, BA - F34
Discloses no financial relationships with commercial entities.

Bryan R. Burnett, MS - B200, E22, LW5
Discloses no financial relationships with commercial entities.

JoAnn Buscaglia, PhD - B91
FBI Laboratory (Employee).

Alice J. Butcher, BSc - A67
Discloses no financial relationships with commercial entities.

John M. Butler, PhD
Thomson Reuters (Discussion of Commercial Products or Services). - W1
National Institute of Standards and Technology (Employee). - LW1, W1

David Buzby, BS - K62, K72
NMS Labs (Employee).

Patrick Buzzini, PhD - W9
Discloses no financial relationships with commercial entities.

Nichole D. Bynum, MS - B76
FLIR Systems, Inc (Discussion of Commercial Products or Services).
National Institute of Justice (Grant Support).

Jennifer F. Byrnes, PhD - A29
University of Hawaii - West O'ahu (Employee).

Joan A. Bytheway, PhD
Discloses no financial relationships with commercial entities. - S2
AceTool, Condor Tool & Knife, Dexter-Russell, Inc, Estwing, HDX, Marshalltown Company, Ryobi (Discussion of Commercial Products or Services). - A123

C

Matthew D. Cain, MD
SAS Institute, Inc (Discussion of Commercial Products or Services). - H109
GitHub, Inc, Oracle Corporation, Twitter Bootstrap Team (Discussion of Commercial Products or Services). - H130

Ismail Çakir, PhD - J4
CTMS, Leica Microsystems (Discussion of Commercial Products or Services).

Roberto Cameriere - G17
Discloses no financial relationships with commercial entities.

Jessica L. Campbell, MS - A81
Ousley, S.D. (Discussion of Commercial Products or Services).

University of Indianapolis (Grant Support).

Janice Canedo
Google, Inc (Discussion of Commercial Products or Services). - C18
Auburn Cyber Research Center (Other Financial/Material Support). – C23

Sarah E. Canty, PhD - A55
Liverpool John Moores University (Employee).

Jodi M. Caple, BS - A71
Perception Lab (Discussion of Commercial Products or Services).

Felice F. Carabellese, MD - I21
Discloses no financial relationships with commercial entities.

Sean Y. Carlson-Greer, BA - A31
Solution Technologies, Inc (Discussion of Commercial Products or Services).

Amy Y. Carney, PhD - W24
Discloses no financial relationships with commercial entities.

Kelsey A. Carpenter, BS
Leica Microsystems (Discussion of Commercial Products or Services). - A24
Discloses no financial relationships with commercial entities. - S2

Mark Carroll, BA - W24
Discloses no financial relationships with commercial entities.

Mariah D. Carson, BS - K29
Quetiapine (Discussion of Unlabeled/Investigational Use of Product/Device).

David O. Carter, PhD - H22
Chaminade University of Honolulu (Employee).

Carlos B. Carvalho, PhD - B60
National Institute of Criminalistics, Brazilian Federal Police (Employee).

Mary E.S. Case, MD - BS1
Discloses no financial relationships with commercial entities.

Rudy J. Castellani, MD - H70
Discloses no financial relationships with commercial entities.

Maria C. Castellanos, MFS - E44
Calumet Specialty Products Partners L.P., Foster + Freeman, Ltd, Nikon, Inc (Discussion of Commercial Products or Services).
Air Force Office of Investigations (Employee).

Michael Cavilla, BA - W11
Discloses no financial relationships with commercial entities.

Giovanni Cecchetto, MD, PhD - H92
Discloses no financial relationships with commercial entities.

Elizabeth N. Celata, MS - A133
Binghamton University (Other Financial/Material Support).

Adam Cervellone, BS - C19
AccessData, Apple, Inc, DELL, Guidance Software, Inc, SANS Institute (Discussion of Commercial Products or Services).

Gürsel Çetin, MD - J4
Discloses no financial relationships with commercial entities.

Selcuk Cetin, MD - H43
Discloses no financial relationships with commercial entities.

Kathryn R. Chabaud, BS - B147
National Institute of Justice (Grant Support).

Eric Chaghouri, MD - I8
Discloses no financial relationships with commercial entities.

Ayako Chan-Hosokawa, MS - K49
NMS Labs (Employee).

Kermit B. Channell II, BS - B25
Discloses no financial relationships with commercial entities.

Carole E. Chaski, PhD
ALIAS Technology, LLC (Discussion of Commercial Products or Services). - D9
Multilingual Parser (Discussion of Unlabeled/Investigational Use of Product/Device). - D9
Discloses no financial relationships with commercial entities. - I38

Muhammad Taimoor Chaudhary, MPhil - E73, K16, K34
Discloses no financial relationships with commercial entities.

Vikram Raj Singh Chauhan, PhD - J12
Discloses no financial relationships with commercial entities.

Heather I. Chen, BA - H100
Discloses no financial relationships with commercial entities.

Susan Cheng, BS - B7
QIAGEN, Inc, Zymo Research (Discussion of Commercial Products or Services).
Cedar Crest College (Other Financial/Material Support).

Jacob L. Cheramie - A110
IBM Corporation, Jantz, R.L/Ousley, S.D. (Discussion of Commercial Products or Services).

Elizabeth Chesna, BS - I30
Discloses no financial relationships with commercial entities.

Linda L. Chezem, JD - F3
Discloses no financial relationships with commercial entities.

Hae Joung Cho - A44
GOM, VATECH America, Z Corporation (Discussion of Commercial Products or Services).

Helen Cho, PhD - A44
GOM, VATECH America, Z Corporation (Discussion of Commercial Products or Services).

Angi M. Christensen, PhD - A1
Discloses no financial relationships with commercial entities.

Sheresa Christopher, PhD - I3, I24
Discloses no financial relationships with commercial entities.

Hee-Sun Chung, PhD - K28
Discloses no financial relationships with commercial entities.

Jennifer D. Churchill, PhD - B54
Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).
UNTHSC (Employee).

Dennis J. Chute, MD - H91
Dutchess County MEO (Employee).

Maria Susana Ciruzzi, PhD - F43
Discloses no financial relationships with commercial entities.

Steven C. Clark, PhD - E17
Discloses no financial relationships with commercial entities.

Jordan L. Clarke, BS - H13
Applied Biosystems, Inc, Promega Corporation, QIAGEN, Inc (Discussion of Commercial Products or Services).
North Carolina Department of Justice (Grant Support).

T. Douglas Clifford, JD - F40
Discloses no financial relationships with commercial entities.

Karen Cline-Parhamovich, DO - K75
Discloses no financial relationships with commercial entities.

Michael D. Coble, PhD - B174
National Institute of Standards and Technology (Employee).

Ashley Cochran, BS - B45
Agilent Technologies (Discussion of Commercial Products or Services).
Ken F. Cohrn, DDS - G21
Discloses no financial relationships with commercial entities.
Federica Collini, MD - H27, I35, S2
Discloses no financial relationships with commercial entities.
John Collins, Jr., MA - S1
Discloses no financial relationships with commercial entities.
Mary B. Collins-Morton, MS
Federal Bureau of Investigation CIRG FBI Academy (Employee). - BS2
Discloses no financial relationships with commercial entities. - W8
Aime Conigliaro, MA - G40
Discloses no financial relationships with commercial entities.
Gerald J. Conlogue, MHS - W18
Quinnipiac University (Employee).
Katie Conners - F8
State of Minnesota (Employee).
Melissa A. Connor, PhD - E18
Onset Computer Corporation (Discussion of Commercial Products or Services).
Kristin K. Cooke, BS - E15
Discloses no financial relationships with commercial entities.
Stuart Cooper, MSc - B96
New Zealand Crown Research Institute ESR, Forensic Science South Australia (Discussion of Commercial Products or Services).
Katie Corcoran, BS - A74
The Department of Defense (Grant Support).
S. Cordner, MB - W16
Discloses no financial relationships with commercial entities.
Tracey S. Corey, MD - K75
Discloses no financial relationships with commercial entities.
Jered B. Cornelison, PhD - H32
Discloses no financial relationships with commercial entities.
Charles R. Cornett, PhD
WiSys Technology Foundation, University of Wisconsin-Platteville (Grant Support). - B73
Discloses no financial relationships with commercial entities. - B156
Brigida Corrieri, MSc - A62
Discloses no financial relationships with commercial entities.
Amanda K. Costello, MS - A92
Solution Technologies, Inc (Discussion of Commercial Products or Services).
Joseph A. Cox, MS - K6
Expertox, Inc (Employee).
Christian Crowder, PhD - A127
Harris County Institute of Forensic Sciences (Employee).
Breanna M. Cuchara - E47
Discloses no financial relationships with commercial entities.
Eugenia Cunha, PhD - A73
Universidade de Coimbra (Employee).
David Cunningham, PhD - B33
IonSense, Inc, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).
Krista Currie, MSc - B211
Discloses no financial relationships with commercial entities.
Trevor E. Curtis, BS - E37
Eternal Tattoo Supply, StarBrite, (Discussion of Commercial Products or Services).
Forensic Sciences Foundation, Inc. Lucas Grant (Grant Support).
Natalia Czado, MS - B2
GE Healthcare Life Sciences, Illumina, Inc, Life Technologies Corporation, QIAGEN, Inc, Sigma-Aldrich Co (Discussion of Commercial Products or Services).
Sam Houston State University (Employee).

D

Corinne D'Anjou, DMD - G32
Discloses no financial relationships with commercial entities.
Gretchen R. Dabbs, PhD - A120
IBM Corporation (Discussion of Commercial Products or Services), Southern Illinois University (Employee).
Ian Dadour, PhD - H118
Discloses no financial relationships with commercial entities.
Nebile Gokce Daglioglu, PhD - K26
Cukurova University (Grant Support).
Richard N. Dalby, PhD - W14
Discloses no financial relationships with commercial entities.
Cristina M. Dalle Grave, DDS - G47
Dennis Babkin, Dexis, Microsoft Corporation (Discussion of Commercial Products or Services).
Matthew J. Danker, BS - E75
Discloses no financial relationships with commercial entities.
William C. Darby, MD - I6, I40
Discloses no financial relationships with commercial entities.
Angela M. Dautartas, MA - A118
National Institute of Justice, United States Department of Justice (Grant Support).
Thomas J. David, DDS - G26
Discloses no financial relationships with commercial entities.
Brent Davis, MD - H75
Mississippi State Medical Examiner's Office (Employee).
Gregory G. Davis, MD - BS3
Jefferson County (Employee).
Lucy A. Davis, BHS - ES1
Discloses no financial relationships with commercial entities.
William M. Davis, PhD - B86
PerkinElmer, Inc, Royal Canadian Mounted Police (Discussion of Commercial Products or Services).
Josep De Alcaraz-Fossoul, PhD - B68
Discloses no financial relationships with commercial entities.
Peter R. De Forest, DCrim - W9
Discloses no financial relationships with commercial entities.
Guilherme H.B. de Miranda - E74
Brazilian Federal Police (Employee).
Ilaria De Vitis, MD - I23
Discloses no financial relationships with commercial entities.
Sara A. Debus-Sherrill - E89
National Institute of Justice (Grant Support).
Summer J. Decker, PhD - A7
IBM Corporation (Discussion of Commercial Products or Services).
Stephanie DeDore, BS - B110
FTI, IntegenX, Inc, NetBio, Inc (Discussion of Commercial

Products or Services).
SNA International (Paid Consultant).

Fabrice F. Dedouit - E70
Discloses no financial relationships with commercial entities.

Audrey Deeken-Draisey, MD - H34
Discloses no financial relationships with commercial entities.

Tania Delabarde, PhD - A79
Discloses no financial relationships with commercial entities.

Yann Delannoy, MD - A22
Discloses no financial relationships with commercial entities.

Dana Delger, JD - F36
Innocence Project (Employee).

John P. Demas, DDS - W5
Trumbull, ICRA Sapphire, Inc (Discussion of Commercial Products or Services).

Gina Dembinski, MS - B61
Life Technologies Corporation, Promega Corporation (Discussion of Commercial Products or Services).

Frank DePaolo, BS - W5
American Airlines, Trumbull, ICRA Sapphire, Inc (Discussion of Commercial Products or Services).
Office of Chief Medical Examiner of the City of New York (Employee).

Rebecca T. DeRienz, MS - K74
Franklin County Coroner's Office (Employee).

Ketaki Deshpande, MS - H18
QIAGEN, Inc (Discussion of Commercial Products or Services).

Betty Layne DesPortes, JD, MS - F3
Discloses no financial relationships with commercial entities.

Sylvain Desranleau, DMD - G33
ThéMA University of Franche (Discussion of Commercial Products or Services).

Kelsey M. DeWitt, BS - B196
Bruker Corporation (Discussion of Commercial Products or Services).
Food and Drug Administration's Forensic Chemistry Center (Employee).

Todd A. Deyne, BsC - E78
IBM Corporation, National Institute of Standards and Technology, Royal Society of Chemistry, Sigma-Aldrich Co., The R Foundation, The Samuel Roberts Noble Foundation (Discussion of Commercial Products or Services).

Shivani Dhaka, MBBS - K20
AIIMS (Employee).

Ciro Di Nunzio, MFS, PhD - B65, B66, H97
Discloses no financial relationships with commercial entities.

Giancarlo Di Vella, MD, PhD
Life Technologies Corporation, Promega Corporation (Discussion of Commercial Products or Services). - B144
Discloses no financial relationships with commercial entities. - I28, H40

Peter J. Diaczuk, BS - B165
Discloses no financial relationships with commercial entities.

James R. Dibble, BS - W22
Discloses no financial relationships with commercial entities.

Allyson K Digmann, BS - B79
Southeast Missouri State University (Other Financial/Material Support).

Hasan Din, MD - H43
Discloses no financial relationships with commercial entities.

Ricardo Jorge Dinis-Oliveira - H3
Discloses no financial relationships with commercial entities.

Lawrence A. Dobrin, DMD - W5
Trumbull, ICRA Sapphire, Inc (Discussion of Commercial Products or Services).

Halis Dokgöz - J9
Grimed (Discussion of Commercial Products or Services, Discussion of Unlabeled/Investigational Use of Product/Device, and Other Financial/Material Support).

Julia A. Dolan, MS - B205
Bureau of Alcohol, Tobacco, Firearms and Explosives (Employee).

Stephanie Domitrovich, JD, PhD - F14, F22
Discloses no financial relationships with commercial entities.

Laura Donato - A40
Discloses no financial relationships with commercial entities.

Robert B.J. Dorion, DDS - G27, G28
Discloses no financial relationships with commercial entities.

Meryle A. Dotson, MA - E32
Discloses no financial relationships with commercial entities.

Kyle C. Doty, BS - B193
National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. (Grant Support).

Barbara Doupe, MSc - B157
The Centre of Forensic Sciences funded the materials used in the project (Employee).

Ana Paula S. Doval - B210
Discloses no financial relationships with commercial entities.

J.C. Upshaw Downs, MD
Discloses no financial relationships with commercial entities. - F13, S2
AMBLIN Entertainment (Discussion of Commercial Products or Services). - BS5

Sara N. Doyle, MD - H107
Harris County Institute of Forensic Sciences (Employee).

Derek M. Draft, DDS - G13
Microsoft Corporation (Discussion of Commercial Products or Services).

Stacy A. Drake, PhD, MPH - E80
Discloses no financial relationships with commercial entities.

Meaghan P. Drumm, BA - K25
Agilent Technologies, BioTage (Discussion of Commercial Products or Services).
Arcadia University (Other Financial/Material Support).

Beatrix Dudzik, PhD - A30
The R Foundation (Discussion of Commercial Products or Services).

Deiter J. Duff, MD - H80
Discloses no financial relationships with commercial entities.

Rebecca F. Dunn - B23
Discloses no financial relationships with commercial entities.

Tim G. Dunn, MS - D30
Discloses no financial relationships with commercial entities.

Jessica B. Dwyer, MD - K66
Allegheny County Medical Examiner's Office (Employee).

R. Gregg Dwyer, MD, EdD - I16
Discloses no financial relationships with commercial entities.

Josiah Dykstra, PhD - W4

Discloses no financial relationships with commercial entities.

E

Glenda M. Easterling, BS - K17
Office of the Chief Medical Examiner, San Francisco (Employee).
Michael D. Eckhardt, MD - H31
Discloses no financial relationships with commercial entities.
Christopher J. Ehrhardt, PhD - B104
National Institute of Justice, Virginia Commonwealth University (Grant Support).
Heidi Eldridge, MS - B162, W12
National Institute of Justice (Grant Support).
Albert A. Elian, MS - K41
Discloses no financial relationships with commercial entities.
George Elias - I10
Discloses no financial relationships with commercial entities.
Kelly M. Elkins, PhD - B8
Lee BioSolutions, Microsoft Corporation, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).
Towson University (Employee).
Sarah Ellingham, MSc - A50
Discloses no financial relationships with commercial entities.
Sarah J. Ellis, MS - S2
Discloses no financial relationships with commercial entities.
Kyleen Elizabeth Elwick, BS - B4
Applied Biosystems, Inc, Eppendorf AG, Life Technologies Corporation, Promega Corporation, ZyGem Corporation, Ltd (Discussion of Commercial Products or Services).
Dakota W. Emery - F33
Agilent Technologies, Branson, Enzo Life Sciences, Inc, Sigma Chemicals (Discussion of Commercial Products or Services).
University of Alaska Fairbanks (Grant Support).
Spice, Synthetic Cannabinoids. (Discussion of Unlabeled/Investigational Use of Product/Device).
Alexandra L. Emmons, MA - A61
University of Tennessee. (Other Financial/Material Support).
Elizabeth A. Erickson, MS - E25
Discloses no financial relationships with commercial entities.
Anders Eriksson, MD, PhD
Discloses no financial relationships with commercial entities. - H51
Elsevier, John Wiley & Sons, Inc, National Library of Medicine, University of Bristol (Discussion of Commercial Products or Services). - H54
Kenyon M. Evans-Nguyen, PhD - W2
BaySpec, Inc, 1st Detect, FLIR Systems, Inc, IonSense, Inc, MassTech, , Microsaic Systems plc, 908 Devices, Prosolia, Inc, Smiths Detection, Torion, Waters Corporation (Discussion of Commercial Products or Services).
The University of Tampa (Employee).
Cynthia L. Evenson, JD - F35
Discloses no financial relationships with commercial entities.

F

Maxwell Christopher Fabricant, JD - F36
Innocence Project (Employee).
Dedouit Fabrice, MD - E41
Discloses no financial relationships with commercial entities.
Paolo Fais, MD - H94
Discloses no financial relationships with commercial entities.
Laura C. Farese, MD - G2
Discloses no financial relationships with commercial entities.
Armin A. Farid, DDS - G56
Discloses no financial relationships with commercial entities.
Amanda L. Farrell, PhD - W21
Discloses no financial relationships with commercial entities.
Davin Faulkner, DMD - W5
Trumbull, ICRA Sapphire, Inc (Discussion of Commercial Products or Services).
Marc Feaster, BS - K19
Draeger (Discussion of Commercial Products or Services).
Office of the Chief Medical Examiner, NC (Employee).
J. Paul Fedoroff, MD - I16
Discloses no financial relationships with commercial entities.
Joseph Ferencz, MD, PhD - I45
Discloses no financial relationships with commercial entities.
Lyndsie N. Ferrara, MS - E26
Duquesne University (Employee).
Renato T. Ferreira de Paranaiba, BA - B57
Affymetrix, Inc, National Library of Medicine, New England Biolabs, Tamura/Stecher/Peterson/Filipski/Kumar, Ratnasingham, S/Hebert, P.D.N., Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).
Brazilian Federal Police (Employee).
Kenneth E. Ferslew, PhD - K75
Discloses no financial relationships with commercial entities.
Christopher Fields, MD - I3, I24
Discloses no financial relationships with commercial entities.
Alejandra Figueroa, BSc - B59
Illumina, Inc, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).
Program Fondecyt (Grant Support).
Marissa J. Finkelstein, MS - K30
Bayer Healthcare Pharmaceuticals, Lilly USA, LLC, Pfizer, Inc, SCIEX, Shimadzu Corporation (Discussion of Commercial Products or Services).
Aegis Sciences Corporation (Other Financial/Material Support).
Sheree J. Finley, MS
Discloses no financial relationships with commercial entities. - H112
National Science Foundation (Grant Support). - H120
Richard H. Fixott, DDS - G29
Discloses no financial relationships with commercial entities.
Jamie N. Fleming, BS - B16
Federal Bureau of Investigation (Other Financial/Material Support).
Martina Focardi - H48, H93
Discloses no financial relationships with commercial entities.
Patricia A. Foley-Melton, PhD - B98
Brenner, C.H., Cybergenetics, Softgenetics, NicheVision, Inc, (Discussion of Commercial Products or Services).
National Institute of Justice, RTI International (Other Financial/Material Support).
Luis Fondebrider, PhD - W16

Discloses no financial relationships with commercial entities.

Jonathan M. Ford, PhD - A7
IBM Corporation (Discussion of Commercial Products or Services).

A.R.W. Forrest, LLM - F12
Discloses no financial relationships with commercial entities.

Matthew F. Fox, MD - H102
Discloses no financial relationships with commercial entities.

Lara Frame-Newell, MA - S2
Discloses no financial relationships with commercial entities.

Darren Franck, MSME - D5
National Institute of Standards and Technology (Discussion of Commercial Products or Services).

Ademir Franco, MSc - G55
AGE Solutions, Cad Cam Technologies, EDF, Maestro, Telecom ParisTech (Discussion of Commercial Products or Services).

Meredith A. Frank, MD - H104
Denver Office of the Medical Examiner (Employee).

Katrin Franke, PhD - W20
Discloses no financial relationships with commercial entities.

Annarita Franza, PhD - I22, I44
Discloses no financial relationships with commercial entities.

Kristen Frederick-Frost, PhD - LW1
National Institute of Standards and Technology (Employee).

Michael Freeman, MD, PhD - H61
National Highway Traffic Safety Administration (Discussion of Commercial Products or Services).

Clare M. Fried, BS - B43
Albrayco Technologies, Inc, SCIEX, WD-40 Company (Discussion of Commercial Products or Services).
Cedar Crest College (Other Financial/Material Support).

Melissa Friscia, MSFS - K9
Waters Corporation (Discussion of Commercial Products or Services).
National Institute of Justice (Employee).
The Center for Forensic Science Research and Education (Grant Support).

John Fudenberg, MBA - W5
Trumbull, ICRA Sapphire, Inc (Discussion of Commercial Products or Services).
Clark County Coroner's Office (Employee).

Sarah M. Furnier, BS - A114
Jantz R.L. /Ousley, S.D. (Discussion of Commercial Products or Services).

Kenneth G. Furton, PhD - S1
Florida International University (Employee).

G

Hallie Gaffney - A93
State University of New York (Grant Support).

Catherine M. Gaither, PhD - A116
Discloses no financial relationships with commercial entities.

Elizabeth A. Gardner, PhD - B82
National Science Foundation (Grant Support).

Luciano Garofano, PhD - E71
Apple, Inc (Discussion of Commercial Products or Services). - B81
Discloses no financial relationships with commercial entities. -E71

Paolo Garofano, MD, PhD - B95
Brenner, C.H., Netherlands Forensic Studio, SCIEG (Discussion of Commercial Products or Services).

Dominic Gascho - H90
Discloses no financial relationships with commercial entities.

Vernon J. Geberth, MS, MPS - W10
CRC Press, LLC (Discussion of Commercial Products or Services).
PHI Investigative Consultants, Inc (Speakers Bureau).

Diana Geli - H73
Immunalysis Corporation (Discussion of Commercial Products or Services).

Steven Geniuk, MS - E16, W7
Discloses no financial relationships with commercial entities.

Rebecca L. George, MA - A65
California State University, Chico (Grant Support).

Charles E. Georget, PhD - G31
Discloses no financial relationships with commercial entities.

Zeno J. Geradts, PhD
Ministery of Security and Justice (Employee). - C2, W20
Canon, Inc, Facebook, Inc, Google, Inc (Discussion of Commercial Products or Services). - C13
Netherlands Forensic Institute (Employee). - C13, S1

Katherine B. Gettings, PhD - W23
Battelle Memorial Institute, Illumina, Inc, Promega Corporation, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).
National Institute of Standards and Technology (Employee).

Melodi Ghui - A21
Discloses no financial relationships with commercial entities.

Dean M. Gialamas, MS - W8
Discloses no financial relationships with commercial entities.

Zac P. Giammarrusco, MS - C14
aTube Downloader, GoPro, Inc, Real Networks, Inc, YouTube, LLC, YTD Downloader, (Discussion of Commercial Products or Services).

Kemper Gibson - B111
Takara Bio, Inc, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).

Georgiana C. Gibson-Daw, MS - B12
Florida International University (Employee).

Mark A. Giffen, Jr., DO - H132
Discloses no financial relationships with commercial entities.

Katelyn M. Gigl, BS - B112
Harris, IntegenX, Inc, NetBio, Inc, Promega Corporation, QIAGEN, Inc, SoftGenetics, LLC, Thermo Fisher Scientifice, Inc, ZyGem Corporation, Ltd (Discussion of Commercial Products or Services).
The Pennsylvania State University (Other Financial/Material Support).
DNA analysis reagents, instruments, software (Discussion of Unlabeled/Investigational Use of Product/Device).

Jack Gilbert, PhD - H127
Discloses no financial relationships with commercial entities.

M.G.F. Gilliland, MD - H135
Discloses no financial relationships with commercial entities.

Rhesa G. Gilliland, MS - W22
Discloses no financial relationships with commercial entities.

Cinzia Gimelli, PsyD, PhD - I15

Discloses no financial relationships with commercial entities.

Simone Gittelson, PhD - B216
Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).
National Institute of Standards and Technology (Employee).

Anna Gitto, JD - F15
Discloses no financial relationships with commercial entities.

Lorenzo Gitto, MD
Discloses no financial relationships with commercial entities.- H66
Facebook, Google, Inc, IG Market, LabLynx, Inc, LinkedIn, Snapchat, Inc, Twitter (Discussion of Commercial Products or Services). - H139

Lindsay Glicksberg - K8
National Institute of Justice (Grant Support).

Claire Glynn, PhD- B5
QIAGEN, Inc, Zymo Research (Discussion of Commercial Products or Services).
University of New Haven (Other Financial/Material Support).

Timothy P. Gocha, PhD - A82
ESRI, IBM Corporation (Discussion of Commercial Products or Services).

Kanya Godde, PhD - A91
University of La Verne (Grant Support).

Francisco Valente Gonçalves, MSc - B22
Marie Curie Early Stage Researcher funded by the European Union (Grant Support).

James Gooch - B181
King's College London, Metropolitan Police Service, UK Home Office (Grant Support).

James F. Goodrich, BDS - G32
Discloses no financial relationships with commercial entities.

Erin M. Gorden, MFS
New England BioLabs (Discussion of Commercial Products or Services). - B179
Illumina, Inc, Kapa Biosystems, New England Biolabs (Discussion of Commercial Products or Services). - W23
AFDIL (Employee). - B177, B179, W23

Christopher J. Gordon, MD - H49
Discloses no financial relationships with commercial entities.

Jan M. Gorniak, DO - H140
IAC Company (Discussion of Commercial Products or Services).

Emily D. Gottfried, PhD - I12
PAR, Inc (Discussion of Commercial Products or Services).

Lynsey F. Gozna, PhD - I19
Discloses no financial relationships with commercial entities.

Silke Grabherr, PhD - H133
Discloses no financial relationships with commercial entities.

Ema H. Graham - B132
Applied Biosytems, Inc, Promega, QIAGEN, Inc, Spectronics Corporation, SPEX SamplePrep (Discussion of Commercial Products or Services and Discussion of Unlabeled/Investigational Use of Product/Device).

Michael A. Graham, MD - H70
Discloses no financial relationships with commercial entities.

Abigail J. Grande, BS - H59
Discloses no financial relationships with commercial entities.

Ignazio Grattagliano, PsyD - I2, I14, I29
Discloses no financial relationships with commercial entities.

Ashley Green, MA - A80
Discloses no financial relationships with commercial entities.

Jacob Griffin, BS - A14
The R Foundation (Discussion of Commercial Products or Services).

Catalin Grigoras, PhD
Alesis, D&M Holdings, Inc, Olympus America, Inc, Philips, Roland Corporation, SanDisk Corporation, Sony Corporation of America, TASCAM, Toshiba America Information Systems, Inc, Zoom Corporation (Discussion of Commercial Products or Services).
University of Colorado Denver (Employee). - C10
GoPro, Inc (Discussion of Commercial Products or Services). - C14

Megan E. Grimes, MFS - B114
Applied Biosystems, Inc, QIAGEN, Inc (Discussion of Commercial Products or Services).
ORISE (Other Financial/Material Support).

Kelly Grisedale, PhD - B10
Illumina, Inc, Life Technologies Corporation, QIAGEN, Inc, University of North Texas Health Science Center (Discussion of Commercial Products or Services).
Western Carolina University (Employee).

Fessessework Guale, DVM - K69
Harris County Institute of Forensic sciences (Employee).

Petur G. Gudmannsson, MD - H51
Discloses no financial relationships with commercial entities.

Richard A. Guerrieri, MS - W23
Battelle Memorial Institute, Promega Corporation, Illumina, Inc (Discussion of Commercial Products or Services). - W23
Battelle Memorial Institute (Employee). - S1, W23

Mark D. Guido, MS - C1
Samsung, Google, Inc (Discussion of Commercial Products or Services).
The MITRE Corporation (Employee).

Mete K. Gulmen, PhD, MD - E14, K26
Discloses no financial relationships with commercial entities.

Chinmoy Gulrajani, MD - I6
Discloses no financial relationships with commercial entities.

Ayse Gulsahi, PhD - G15
Discloses no financial relationships with commercial entities.

Pramod Gumpeni, MD - H111
Dupont (Discussion of Commercial Products or Services).
Harris County Institute of Forensic Sciences (Employee).

Zhaoming Guo, MD - H36, H65, H141
Discloses no financial relationships with commercial entities.

Avneesh Gupta, MD - H67
Discloses no financial relationships with commercial entities.

Murat Serdar Gürses, MD
Uludag University Network (Discussion of Commercial Products or Services). - H37
Discloses no financial relationships with commercial entities. - H43

Torfinn Gustafsson, BM - H55
Discloses no financial relationships with commercial entities.

H

Melinda Hacker, DDS - G6

Discloses no financial relationships with commercial entities.

Jeffery Hackett, PhD - K41
Discloses no financial relationships with commercial entities.

Kathryn H. Haden-Pinneri, MD - E34
Discloses no financial relationships with commercial entities.

Amanda L. Haggerty, BS - E20
Agilent Technologies, AMS, Inc, CTC Analytics (Discussion of Commercial Products or Services).

Sarah V. Hainsworth, PhD - D1, D14
University of Leicester (Employee).

Amanda R. Hale, MA
North Carolina State University (Employee). - S1
Discloses no financial relationships with commercial entities. - S2

Adam B. Hall, PhD
Cumberland Farms, Gulf Corporation, IonSense, Inc, Irving Corporation, Sunoco Corporation, Shell Corporation, (Discussion of Commercial Products or Services). - B42
Agilent Technologies, Field Portable, IonSense, Inc, 908 Devices, SCIEX (Discussion of Commercial Products or Services). - W2
Northeastern University (Employee). - B42, W2

Ashley Hall, PhD - B137
Life Technologies Corporation, Promega Corporation (Discussion of Commercial Products or Services).
University of Nebraska-Lincoln (Employee).

Jacob R. Hall - C15
Apple, Inc (Discussion of Commercial Products or Services).

Christine L. Halling, MS - E64
Discloses no financial relationships with commercial entities.

Kristine Hamann, JD - F6
Discloses no financial relationships with commercial entities.

Austin Hancock, BS - C18
Google, Inc (Discussion of Commercial Products or Services).
Auburn University (Other Financial/Material Support).

Randy L. Hanzlick, MD - H70
Discloses no financial relationships with commercial entities.

Brett E. Harding, MBA - E59
Discloses no financial relationships with commercial entities.

Laurel A. Hardy, BS - B35
Restek Corporation (Discussion of Commercial Products or Services).

Heather L. Harris, MFS, JD - F40
Discloses no financial relationships with commercial entities.

Howard A. Harris, JD, PhD - E65
Discloses no financial relationships with commercial entities.

Kristen Hartnett-McCann, PhD - A93
SOFA Grant, State University of New York Scholarly and Creative Activity Grant (Grant Support).

Gary M. Hatch, MD - A1
New Mexico Office of the Medical Investigator (Employee).

Kino Hayashi, MD - H46
Discloses no financial relationships with commercial entities.

Donald Hayden, MFS
Discloses no financial relationships with commercial entities. - E16, W7

Christina G. Hayes, BS
Discloses no financial relationships with commercial entities. - S2
St. Louis Metropolitan Police Department (Employee) - S1

Jonathan Hayes, MD - H70
Discloses no financial relationships with commercial entities.

Courtney Head, MS - F31
Federal Bureau of Investigation (Discussion of Commercial Products or Services).
Houston Forensic Science Center (Employee).

Joseph T. Hefner, PhD - A28
Discloses no financial relationships with commercial entities.

Dagmar Heinrich, PhD - E60
EPSRC Grant at UCL SECReT, Secure Societies Institute, University of Huddersfield (Grant Support).

Donna J. Hellwinkel, DDS - G39
Google, Inc (Discussion of Commercial Products or Services).
Washoe County Medical Examiner's Office (Paid Consultant).

Jeanet Hendrikse, MSc - B148
Discloses no financial relationships with commercial entities.

Matthew T. Henshon, AB, JD - W20
Discloses no financial relationships with commercial entities.

Martin Herman, PhD - C16
National Institute of Standards and Technology (Employee).

Edward E. Herschaft, DDS - W5
Office of the Chief Medical Examiner City of New York (Discussion of Commercial Products or Services).

Kaitlyn E. Hess, BS - K38
Restek Corporation, SCIEX, Shimadzu Corporation (Discussion of Commercial Products or Services).
Bio-SPME fiber stationed in pipet tippet (Discussion of Unlabeled/Investigational Use of Product/Device).
Cedar Crest College (Other Financial/Material Support).

Charles M. Heurich, MFS - B212
United States Department of Justice/National Institute of Justice (Employee).

Terry-Dawn Hewitt, LLM - F41
Discloses no financial relationships with commercial entities.

Maureen Hickman, MS - B50
Illumina, Inc (Discussion of Commercial Products or Services).
Agilent Technologies, Illumina, Inc (Discussion of Unlabeled/Investigational Use of Product/Device).
National Institute of Justice (Grant Support).

Jack Hietpas, PhD - B83
National Institute of Standards and Technology (Discussion of Commercial Products or Services).
Oak Ridge Institute for Science and Education, Federal Bureau of Investigation (Grant Support).

Jennifer L. Higginbotham, MFS - W23
CLC bio, Illumina, Inc, New England BioLabs (Discussion of Commercial Products or Services).
Armed Forces DNA Identification Laboratory (Employee).

Diana Ho - F26
Office of the Chief Medical Examiner City of New York (Employee).

Jacob Hock - B146
Discloses no financial relationships with commercial entities.

Jacob E. Hoerter - H64
FUTEK Advanced Sensor Technology, Inc, Omega, Inc, (Discussion of Commercial Products or Services).

FUTEK Advanced Sensor Technology, Inc, instruNet, Omega, Inc, SENSIT Technologies (Discussion of Unlabeled/Investigational Use of Product/Device).

Janne A. Holmgren, PhD - E67
Discloses no financial relationships with commercial entities.

Anastasia Holobinko, MS - A64
Discloses no financial relationships with commercial entities.

Brian J. Holoyda, MD - I33
Discloses no financial relationships with commercial entities.

Thomas J. Holt, PhD - E58
National Institute of Justice (Grant Support).

Daniel M. Honig, PE - D7
RISA Technologies, LLC (Discussion of Commercial Products or Services).

Jurian A. Hoogewerff, PhD
University of Canberra (Employee). - B126, B201

Mary F. Horvath, MFS - W4
Discloses no financial relationships with commercial entities.

Max M. Houck, PhD - F7
Discloses no financial relationships with commercial entities.

Philip E. Houldsworth, MSc - A121
Discloses no financial relationships with commercial entities.

James Hoult, MS - B44
Nippon Soda Co., Ltd (Discussion of Commercial Products or Services).

Rachel M. Houston, BS - B190
Life Technologies Corporation, QIAGEN, Inc (Discussion of Commercial Products or Services).
Sam Houston State University (Employee).

Julie A. Howe, MBA - E17
Discloses no financial relationships with commercial entities.

Anthony W. Hudson, BS - A51
Discloses no financial relationships with commercial entities.

Marilyn A. Huestis, PhD
Discloses no financial relationships with commercial entities. - W16
National Institutes of Health, IRP, National Institute on Drug Abuse (Employee). - K54

Lurena A. Huffman, BS - W21
Discloses no financial relationships with commercial entities.

Ted R. Hunt, JD - F3
Discloses no financial relationships with commercial entities.

Cheryl D. Hunter - S2
Discloses no financial relationships with commercial entities.

Ja'Neisha Hutley, MS - S2
Discloses no financial relationships with commercial entities.

James B. Hyzer, PhD
Discloses no financial relationships with commercial entities. - D23, D25

I

Lavinia Iancu, PhD - H114
Discloses no financial relationships with commercial entities.

Samiah Ibrahim, BSc - J17
Napkin Forever (Discussion of Commercial Products or Services).

Kena Ihle, BA - A24
Leica Microsystems (Discussion of Commercial Products or Services).

Nahyok Im, PhD - A44
GOM, VATECH America, Z Corporation (Discussion of Commercial Products or Services).

Ayesha Imtiaz, MS - J10
Discloses no financial relationships with commercial entities.

Francesca Indorato, MD - K12
Discloses no financial relationships with commercial entities.

Eric A. Ingle, BA - K52
Office of the Chief Medical Examiner, San Francisco (Employee).

Megan E. Ingvoldstad, PhD - A99
Discloses no financial relationships with commercial entities.

Joseph Insana - B152
Discloses no financial relationships with commercial entities.

Lorna C. Irish, BSc - E61
University of Huddersfield (Grant Support).

Mariyam I. Isa, BS - A78
Dassault Systèmes (Discussion of Commercial Products or Services).
National Science Foundation (Grant Support).

Carolyn V. Isaac, PhD - H28
Discloses no financial relationships with commercial entities.

Marilyn Isaacks, BA - A75
Texas State University Research Scholarship (Grant Support).

Daniel S. Isenschmid, PhD
Randox Laboratories, Ltd (Discussion of Commercial Products or Services) - K55
NMS Labs (Employee) - K55, W19

J

Esther Jack, MBBCh
Discloses no financial relationships with commercial entities. - H1
Lucas Grant from the American Academy of Forensic Sciences (Grant Support) - H25

Christian Jackowski, MD, EMBA - H42
Discloses no financial relationships with commercial entities.

David S. Jackson, BS - B34
United States Food and Drug Administration Forensic Chemistry Center (Employee).

George F. Jackson, PhD - K5
Discloses no financial relationships with commercial entities.

Glen P. Jackson, PhD - B94
National Institute of Justice (Grant Support).

Megan L. Jackson, BS - B55
Agilent Technologies, Integrate DNA Technologies, Inc, Kapa Biosystems, Promega Corporation, QIAGEN, Inc, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).
Oak Ridge Institute for Science and Education, Federal Bureau of Investigation (Grant Support).

Monica Jacobs - K2
Discloses no financial relationships with commercial entities.

Yu Ryang Jang, PhD - A44
GOM, VATECH America, Z Corporation (Discussion of Commercial Products or Services).

Kimberly E. Janssen - H89
Office of Chief Medical Examiner, NC (Employee).

Brian L. Janysek, MFS - W7
Department of Defense Office of Inspectors General (Employee).
Hannah C. Jarvis, MRCS - H79
Discloses no financial relationships with commercial entities.
Alexander Jason, BA - E94
Discloses no financial relationships with commercial entities.
Shashi K. Jasra, PhD - H86
Discloses no financial relationships with commercial entities.
Gulnaz T. Javan, PhD
National Science Foundation (Grant Support) - H122, H123
Roger Jefferys, BS - B30
ARCUS, Inc, Beemiller Manufacturing Facility, Forensic Technology International, Kel-Tec CNC Industries, Inc, Norsys Software Corp., RStudio, Ruger & Co., Inc, SCCY Industries, LLC, Springfield Armory, Sturm, Taurus International MFG, Inc USA (Discussion of Commercial Products or Services).
West Virginia University (Employee).
Brad Jenkins, MS - B174
Cybergenetics (Discussion of Commercial Products or Services).
Virginia Department of Forensic Science (Employee).
Jeffrey M. Jentzen, MD - BS1, F14, H60
Discloses no financial relationships with commercial entities.
Yangseung Jeong, PhD - A2
William M. Bass Endowment — Forensic Anthropology Center-University of Tennessee (Grant Support).
Donald J. Johnson, MS - B194
QIAGEN, Inc (Discussion of Commercial Products or Services).
Erica N. Johnson, BA - K23
BioTage, Waters Corporation (Discussion of Commercial Products or Services).
Arcadia University (Other Financial/Material Support).
Mark E. Johnson, PhD - W6
National Institute of Justice (Grant Support).
Melissa A. Johnson, BA - K42
Discloses no financial relationships with commercial entities.
Jude L. Jokwi, MA - F2
Discloses no financial relationships with commercial entities.
Andrea L. Jones, BS - K21
GE Healthcare, SCIEX, Shimadzu Corporation, Ultra (Discussion of Commercial Products or Services).
Cedar Crest College (Other Financial/Material Support).
John P. Jones II, MBA - B40
ASTM International (Discussion of Commercial Products or Services).
National Institute of Standards and Technology and Organization of Scientific Area Committees Affairs (Employee).
Eunah Joo, MS - E91
National Forensic Service (Grant Support).
Kyla M. Jorgenson, MSc - H110
Discloses no financial relationships with commercial entities.
Chelsey A. Juarez, PhD - A3
Jantz, R.L./Ousley, S.D. (Discussion of Commercial Products or Services).
Go-Un Jung, BS - A90
ISTI – CNR, Robert McNeel & Associates, The University of Manchester, Wolfram Research, Inc (Discussion of Commercial Products or Services).
National Research Foundation of Korea and Global PhD Fellowship Program — National Research Foundation- The Ministry of Education, Science and Technology (Grant Support).
Emily Junkins, BS - H115
United States Air Force Research Laboratory (Grant Support).

K

Abuzar Kabir, PhD - B197
Discloses no financial relationships with commercial entities.
Sherri L. Kacinko, PhD
NMS Labs (Discussion of Commercial Products or Services and Employee). - K46, W2
Cynthia Kaeser, MS - B131
Lawrence Livermore National Laboratory (Employee).
Hidetoshi Kakuda - B67
Matrox Electronic Systems, Ltd, Quantum Composers, Inc (Discussion of Commercial Products or Services)
National Police Agency (Grant Support).
Brooke W. Kammrath, PhD
Malvern Instruments, Ltd (Discussion of Commercial Products or Services) - B17
University of New Haven (Employee) - B17, B125
Kelly R. Kamnikar, BS - A106
National Institutes of Health, The R Foundation (Discussion of Commercial Products or Services).
A. Bakarr Kanu, PhD - B70
Winston-Salem State University (Employee and Grant Support).
Fabian Kanz, PhD - A56
Discloses no financial relationships with commercial entities.
Hakan Kar, MS - J9
Grimed, Ltd (Discussion of Commercial Products or Services, Discussion of Unlabeled/Investigational Use of Product/Device, and Other Financial/Material Support).
Hannah A. Kastenbaum, MD - H88
Discloses no financial relationships with commercial entities.
Zuzanna Kazmierczyk, BS - J16
Discloses no financial relationships with commercial entities.
Denise C. Kellaher - I17
Discloses no financial relationships with commercial entities.
Caroline Machal Kelley, BS - B24
United States Food and Drug Administration's Forensic Chemistry Center (Employee).
Jan S. Kelly, BA
Discloses no financial relationships with commercial entities. - J8, J20
LaTrobe University, Skill-Task Training Assessment & Research, Inc - J14
Michael W. Kenyhercz, PhD - A88
R Core Development Team, Joseph Hefner (Discussion of Commercial Products or Services).
Meghan S. Kessler, DO - H72
SPEware Corporation (Discussion of Commercial Products or Services).
Marisa Teal Ketchum, BS - B3

Applied Biosystems, EMD Millipore, QIAGEN, Inc (Discussion of Commercial Products or Services).

Parul Khare, MSc - G8
Adobe Systems Incorporated (Discussion of Commercial Products or Services).

Kazuhiko Kibayashi, MD
Discloses no financial relationships with commercial entities. - K24
Bayer AG (Discussion of Commercial Products or Services). - H45

Christopher Kiefer, MD - H30
Montgomery County Coroner's Office (Employee).

Dong-Ho Eddie Kim, BSc
Discloses no financial relationships with commercial entities. - A6
IBM Corporation, Materialise (Discussion of Commercial Products or Services). - A39

Eunmi Kim, PhD - K4
Busan Institute, National Forensic Service (Employee).

Hyung Seok Kim, PhD - H14
Discloses no financial relationships with commercial entities.

Keli L. King - H9
National Institute of Justice (Grant Support).

Rebecca King, MS - A27
Mercyhurst Archaeological Institute, Immersion, North Carolina State University, Jantz, R.L./Ousley, S.D. (Discussion of Commercial Products or Services).
Boston University School of Medicine (Other Financial/Material Support).

Juliet Kinyua, MSc - B28
Agilent Technologies, Phenomenex, Inc (Discussion of Commercial Products or Services)
EU International Training Network SEWPROF - Marie Curie Grant (Grant Support).

Alexandra R. Klales, PhD
Discloses no financial relationships with commercial entities. - A124
National Institute of Justice (Grant Support). - A72

Natasha M. Knack, BA - I16
Discloses no financial relationships with commercial entities.

Sandra Koch, MS - B89
Discloses no financial relationships with commercial entities.

Whitney A. Kodama, BA - H128
Illumina, Inc, MO BIO Laboratories, Inc (Discussion of Commercial Products or Services).
National Institute of Justice (Grant Support).

Constantine Konstantakis, BA - K43
Discloses no financial relationships with commercial entities.

Roger G. Koppl, PhD - B203
Discloses no financial relationships with commercial entities.

Andrew C. Koutrakos, MS - B17
Malvern Instruments, Ltd (Discussion of Commercial Products
or Services).

Dan Krane, PhD
Applied Biosystems (Discussion of Commercial Products or Services). - B143, F28
Alcotest (Discussion of Commercial Products or Services). - F39

Kelly Kraus, BS - E31
Discloses no financial relationships with commercial entities.

Kewal Krishan, PhD
Discloses no financial relationships with commercial entities. - A8, A38
IrfanViewMATLAB (Discussion of Commercial Products or Services). - C20

Robert Kronstrand, PhD- K65, K75
Discloses no financial relationships with commercial entities.

Brianna Kroon - B37
Discloses no financial relationships with commercial entities.

Melissa K. Kuhn - A113
Discloses no financial relationships with commercial entities.

Kevin P. Kulbacki, MSFS - J11
Discloses no financial relationships with commercial entities.

Kelley Kulick, JD - F27
Discloses no financial relationships with commercial entities.

Stephanie Kumor, MA - K56
NMS Labs (Employee).

Priyanka Kushwaha, MS - H19
Glomics Incorporation (Discussion of Commercial Products or Services).

Aaron R. Kuzel, BS - A68
Discloses no financial relationships with commercial entities.

Kelsey Kyllonen, MA - A42
Oak Ridge Institute for Science and Education, Visiting Scientist Program (Other Financial/Material Support).

L

Ericka N. L'Abbe, PhD - A104
University of Pretoria (Employee).
National Research Foundation Research Grant, South Africa (Grant Support).

Laura M. Labay, PhD - K51
Sanofi-Aventis S.A. (Discussion of Commercial Products or Services).
NMS Labs (Employee).

Anita Lal, MD - H68
Discloses no financial relationships with commercial entities.

Jack N. Lane, MS - B62
Dynex Technologies (Discussion of Commercial Products or Services).

Natalie R. Langley, PhD - A49
Jantz R.L./Ousley, S.D. (Discussion of Commercial Products or Services).
Lincoln Memorial University (Grant Support).

Patrick E. Lantz, MD - W17
Discloses no financial relationships with commercial entities.

Bobby L. LaRue, Jr., PhD - H121
IntegenX, Inc (Discussion of Commercial Products or Services).
University of North Texas Health Science Center (Employee).

Eric F. Law, BS - B30
ARCUS, Inc, Beemiller Manufacturing Facility, Forensic Technology International, Kel-Tec CNC Industries, Inc, Norsys Software Corp., SCCY Industries, LLC, Springfield Armory, Sturm, Ruger & Co., Inc, Taurus International Manufacturing, Inc (Discussion of Commercial Products or Services).

West Virginia University (Employee).

Simon Lax - H124
Sloan Foundation (Grant Support).

Tiffany R. Layne, BS - B187
BIO-RAD Laboratories, Inc, Life Technologies Corporation, Q-Lab, Inc, QIAGEN, Inc, Quanta BioSciences, Inc, Roche (Discussion of Commercial Products or Services).

Erwan Le Garff, MD - G18
Discloses no financial relationships with commercial entities.

Marc A. LeBeau, PhD - BS3
Federal Bureau of Investigation (Employee).

Helene N. LeBlanc, PhD - E87
General Motors Company, Volkswagen of America, Inc (Discussion of Commercial Products or Services).
Natural Science and Engineering Research Council (Grant Support).

Zo-dee Ledger - E79
Discloses no financial relationships with commercial entities.

Igor K. Lednev, PhD
The Mathworks, Inc (Discussion of Commercial Products or Services). - E86
National Institute of Justice, Office of Justice Programs, United States Department of Justice (Grant Support). - B193, E86

F.L. Jim Lee, Jr., MS - J2, J15
Foster + Freeman, Ltd (Discussion of Commercial Products or Services and Employee).

Unsil Lee, MS - E90
Discloses no financial relationships with commercial entities.

Christina A. Leija, MS - E69
Discloses no financial relationships with commercial entities.

Samuel J. Leistedt, MD, PhD - I36
Discloses no financial relationships with commercial entities.

Eric Lemaire, MD - H71
Discloses no financial relationships with commercial entities.

Nikolas P. Lemos, PhD - S2, W19
Discloses no financial relationships with commercial entities.

Ashton D. Lesiak - B122
JEOL, Ltd (Discussion of Commercial Products or Services).
University at Albany-SUNY Presidential Initiatives Fund Grand for Forensic Sciences and Cybersecurity (Grant Support).

Iana Lesnikova, MD, PhD - H7
Independent Forensics, Hologic (Discussion of Commercial Products or Services).

Mark M. LeVaughn, MD - H75
Mississippi State Medical Examiner's Office (Employee).

Carolyn Lewis, BS - B183
Agilent Technologies, Illumina, Inc, Life Technologies Corporation, New England BioLabs, QIAGEN, Inc, Quanta BioSciences Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).
Virginia Commonwealth University (Employee).

Ling Li, MD - H36, H141
Discloses no financial relationships with commercial entities.

Emily Lichtenberger, BS - B155
Agilent Technologies, Office Depot, Inc, Sharpie, Sigma-Aldrich Co., Whatman, Inc (Discussion of Commercial Products or Services).
Nelson Vinueza, North Carolina State University (Grant Support).

Laura L. Liptai, PhD
Free Agent BMX (Discussion of Commercial Products or Services). - D19
BioMedical Forensics (Employee). - D19
Discloses no financial relationships with commercial entities. - W20

Kimberly Litterell, BS - I9
Discloses no financial relationships with commercial entities.

Ning Liu, MA - J3
Hewlett-Packard Development Company, LP (Discussion of Commercial Products or Services).
NMS Labs (Employee).

Barry K. Logan, PhD
NMS Labs (Discussion of Commercial Products or Services and Employee) - K48
NMS Labs (Employee) - BS3

Sarah Long, BS - H58
Discloses no financial relationships with commercial entities.

Kelsey Longe, BS - K63
Perkin Elmer, Inc (Discussion of Commercial Products or Services).
Aegis Sciences Corporation (Employee).

Kaitlyn A. Lopez - A91
Discloses no financial relationships with commercial entities.

Michael M. Losavio, JD - W22
Discloses no financial relationships with commercial entities.

Nicolene Lottering, BS - A84
Discloses no financial relationships with commercial entities.

Kevin M. Lougee, DO - A115
Denver Office of the Medical Examiner (Employee).

Jennifer C. Love, PhD - A103
Discloses no financial relationships with commercial entities.

Landa S. Low, JD - D19
Free Agent BMX (Discussion of Commercial Products or Services).
California Department of Transportation/Legal (Employee).

Jason Gene Lozano, MD - H41
Discloses no financial relationships with commercial entities.

Micheline Lubin, MD - H29
King County (Employee).

Douglas M. Lucas, DSc - W9
Discloses no financial relationships with commercial entities.

Victoria S. Lucas, PhD - G5
Discloses no financial relationships with commercial entities.

Marcella Auxiliadora de Melo Lucena, MS - B158
CBC Brazil, Glock, Inc (Discussion of Commercial Products or Services).

Ira S. Lurie, PhD
Waters Corporation (Discussion of Commercial Products or Services). - B127
Perkin Elmer, Inc (Discussion of Commercial Products or Services). - K58
National Institute of Justice, Perkin Elmer, Inc, George Washington University (Grant Support). - B127
Perkin Elmer, Inc, George Washington University (Grant Support). - K58

Vincenzo Lusa, JD - I22, I44
Discloses no financial relationships with commercial entities.

James R. Lyle, PhD - C6

Discloses no financial relationships with commercial entities.

M

John Mabry, JD - E93
Discloses no financial relationships with commercial entities.
Donna M. MacGregor, MSc
3D Systems, Inc, Skeletal Biology and Forensic Anthropology Research Laboratory (Discussion of Commercial Products or Services). - L2
Queensland University of Technology and Australian Army (Employee). - E23
Queensland University of Technology and Queensland Police Service (Employee). - L2
Teresa Magalhães, PhD - H3, I1
Discloses no financial relationships with commercial entities.
Adela S. Magallanes, BS - H88
Discloses no financial relationships with commercial entities.
Paola A. Magni, PhD - H12
Discloses no financial relationships with commercial entities.
Christopher A. Maier, MA - A86
University of Nevada — Reno Graduate Student Research and Travel Grant (Grant Support).
Heli Maijanen, PhD - A9
Solution Technologies, Inc (Discussion of Commercial Products or Services).
Susan Makar, MA - W1
Thomson Reuters (Discussion of Commercial Products or Services).
National Institute of Standards and Technology (Employee).
Amanda Malanowski, BS - W1
Thomson Reuters (Discussion of Commercial Products or Services).
National Institute of Standards and Technology (Employee).
Katherine F. Maloney, MD - H108
Erie County Medical Examiner's Office (Employee).
Sergey Mamedov, PhD - B84
Discloses no financial relationships with commercial entities.
Holland Maness, DMD - G26
Discloses no financial relationships with commercial entities.
Michael Marciano, MS - B99
Life Technologies Corporation, Promega Corporation (Discussion of Commercial Products or Services).
National Institute of Justice (Grant Support).
Ioan Marginean, PhD - B159
Perkin Elmer, Inc (Discussion of Commercial Products or Services and Discussion of Unlabeled/Investigational Use of Product/Device).
Pierre A. J-L. Margot, PhD - W9
Discloses no financial relationships with commercial entities.
Luisa Marinho, MSc - A23
Department of Archaeology Graduate Travel Grants (Other Financial/Material Support).
Daniel Marion, Jr., PhD - E4
Discloses no financial relationships with commercial entities.
Nicholas Márquez-Grant, PhD - A62
Discloses no financial relationships with commercial entities.
Charla Marshall, PhD
New England BioLabs (Discussion of Commercial Products or Services). - B179
Armed Forces DNA Identification Laboratory (Employee). - B179, W23
Judy Y. Marshall, DMD - G34
Discloses no financial relationships with commercial entities.
Lucas Marshall, MS - K68
Discloses no financial relationships with commercial entities.
Shirley Marshall - F24
Teesside University Staff Development (Other Financial/Material Support).
Pablo Martinez-Escauriaza - E81
Discloses no financial relationships with commercial entities.
Luca Massaro, MD - E85, F1, I31
Discloses no financial relationships with commercial entities.
Evan Matshes, MD - W3, W11
Discloses no financial relationships with commercial entities.
Sabrina Mauf - I5
Discloses no financial relationships with commercial entities.
Filipe Gabriel B. Mauricio, MSc - B151
Higher Education Personnel Improvement Coordination (Grant Support).
Allison Mautone, MD - H62
Tarrant County Medical Examiner's Office (Employee).
Edward Mazuchowski II, MD, PhD
Discloses no financial relationships with commercial entities. - W8
United States Air Force Office of the Armed Forces Medical Examiner (Employee). - H143
Thomas C. McAndrew, BA - W10
Practical Homicide Investigation (Discussion of Commercial Products or Services).
Brittany S. McClain, BA - A96
Texas State University (Other Financial/Material Support).
Carl R. McClary, BA - J7
Discloses no financial relationships with commercial entities.
Hailey Mcclenon - H21
QIAGEN, Inc, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).
BLAST (Basic Local Alignment Search Tool), Geneious Pro Software (Discussion of Unlabeled/Investigational Use of Product/Device).
Brandi C. McCleskey - E6, H76
Discloses no financial relationships with commercial entities.
Soraya McClung - B25
Discloses no financial relationships with commercial entities.
Kyle A. McCormick, MA - A69
Discloses no financial relationships with commercial entities.
Chloe P. McDaneld, BS - A19
Grady Early Grant (Grant Support).
Jennifer L. McDowell, MSc - A129
University of Otago, New Zealand (Grant Support).
Michael D. McDowell, MS - D26
Discloses no financial relationships with commercial entities.
Mary C. McKiel, PhD - ES1
Discloses no financial relationships with commercial entities.
Timothy P. McMahon, PhD - W23
American Registry of Pathology for the Armed Forces DNA Identification Laboratory-Armed Forces Medical Examiner System (Employee).
James F. McNulty, Jr., JD - F10
Discloses no financial relationships with commercial entities.

Lauren Alyssa Meckel, BS
Discloses no financial relationships with commercial entities. - A98
Texas State University (Employee). - A60
Mary S. Megyesi, PhD - A4
Joint POW/MIA Accounting Command-Central Identification Laboratory (Employee).
Andria H. Mehltretter, MSFS - B88
Discloses no financial relationships with commercial entities.
Kelly A. Meiklejohn, PhD - B189
Kapa Biosystems, Inc, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).
Oak Ridge Institute for Science and Education/Federal Bureau of Investigation (Grant Support).
Judy Melinek, MD - L1
Scribner (Discussion of Commercial Products or Services).
Lisa Mertz, MS - B211
Discloses no financial relationships with commercial entities.
Vadim Mesli, MD - H16
Discloses no financial relationships with commercial entities.
Jessica L. Metcalf, PhD - H125
Earth Microbiome Project, Greengenes Database Consortium, Illumina, Inc, MOBIO Laboratories, Inc, QIIME (Discussion of Commercial Products or Services).
Roger D. Metcalf, JD
Discloses no financial relationships with commercial entities. - G22
Tarrant County (Employee). - G23
Suzanne Miles, BS - E56
Utah Bureau of Forensic Services (Employee).
Keith W. Miller, PhD - W22
Discloses no financial relationships with commercial entities.
Raymond G. Miller, DDS - G37, S2
Discloses no financial relationships with commercial entities.
James Millette, PhD - D16
R.J. Reynolds Tobacco Company (Discussion of Commercial Products or Services).
Chris Milroy, MD, LLB - W11
Discloses no financial relationships with commercial entities.
Jisook Min - E.33, E77
Discloses no financial relationships with commercial entities.
T.J. Mitchell, BA - L1
Scribner (Discussion of Commercial Products or Services).
Linton Mohammed, PhD - S2
Discloses no financial relationships with commercial entities.
Mohd Yusmiaidil P. Mohd Yusof, MS - G14
RStudio (Discussion of Commercial Products or Services).
Amanda L.A. Mohr, MSFS - K60
Waters Corporation (Discussion of Commercial Products or Services).
National Institute of Justice and The Center for Forensic Science Research and Education (Grant Support).
Angela S. Mohrhaus, BS - B74
Discloses no financial relationships with commercial entities.
Mehdi Moini, PhD
SCIEX (Discussion of Commercial Products or Services) - B123
Pfizer, Inc, SCIEX, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services). - B129N
George Washington University (Employee). - W20
National Science Foundation (Grant Support). - B123, B129
Ilaria Monico, MS - H47
Discloses no financial relationships with commercial entities.
David S. Moore, MEd - J18
Discloses no financial relationships with commercial entities.
Jennifer A. Moore, DMD - G9
Discloses no financial relationships with commercial entities.
Katherine N. Moore, MS
ALFRED Database, FROG-kb Database (Discussion of Commercial Products or Services). - B141
National Institute of Justice (Grant Support). - B141
National Forensic Laboratory Information System (Other Financial/Material Support). - B29
Melissa D. Moore, BS - B134
Paternity Testing Corporation, QIAGEN, Inc, Tecan Schweiz AG, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).
Stephen L. Morgan, PhD
Discloses no financial relationships with commercial entities. - B170
National Institute of Justice (Grant Support). - B185
Keith B. Morris, PhD - B30
ARCUS, Inc, Beemiller Manufacturing Facility, Forensic Technology International, Kel-Tec CNC Industries, Inc, Norsys Software Corp., RStudio, SCCY Industries, LLC, Springfield Armory, Sturm, Ruger & Co., Inc, Taurus International MFG, Inc (Discussion of Commercial Products or Services).
Department of Defense, West Virginia University (Grant Support).
Reanna J. Morris - A105
Discloses no financial relationships with commercial entities.
Robert J. Morton, MS - BS2
Discloses no financial relationships with commercial entities.
Sharon K. Moses, PhD - E66
Northern Arizona University (Employee).
Thaddeus Mostowtt, MFS - K10
Discloses no financial relationships with commercial entities.
Melissa Mourges, JD - F25, G22
Discloses no financial relationships with commercial entities.
Ashraf Mozayani, PharmD, PhD - F2
Discloses no financial relationships with commercial entities.
Nirvani Mujumdar, MS - B149
Discloses no financial relationships with commercial entities.
Marzena H. Mulawka, MFS - E84
National Institute of Justice (Grant Support).
Dawn M. Mulhern, PhD - W16
Discloses no financial relationships with commercial entities.
Diana Mullis, MD - I3, I24
Discloses no financial relationships with commercial entities.
Shin Muramoto, PhD - B198
Discloses no financial relationships with commercial entities.
Audrey Murchland, BS - A32
Baylor University Undergraduate Research and Scholarly Achievement Small Grant Award (Grant Support).
Lisa Murphy, MCA - I16
Discloses no financial relationships with commercial entities.
Patrick A. Murray, DDS - G38
Maryland Responds Medical Reserve Corps to the Office of the Chief Medical Examiner (Other Financial/Material

Support).

N

Gary H. Naisbitt, PhD - E2
GoPro, Inc (Discussion of Commercial Products or Services).
Marcela Najarro, MFS - E57
Morpho Detection (Discussion of Commercial Products or Services).
Rebecca Najera, DO - I26
Discloses no financial relationships with commercial entities.
Ken-ichiro Nakao - K24
Discloses no financial relationships with commercial entities.
Martin Nau, MD - I11
Discloses no financial relationships with commercial entities.
Andrew Neal, MS - W4
Discloses no financial relationships with commercial entities.
Christina M. Neal, MS - W23
Armed Forces DNA Identification Laboratory (Employee).
Klaus C. Neudecker, MD - W21
Discloses no financial relationships with commercial entities.
Peter Neufeld, JD - F21
Discloses no financial relationships with commercial entities.
Kayla M. Neuman, MS - K44
AIT Laboratories, NMS Labs (Discussion of Commercial Products or Services).
Wisconsin State Laboratory of Hygiene (Employee).
Tara L. Newcomb, MS
Discloses no financial relationships with commercial entities. - G1
Aribex, DENTSPLY International, Patterson Companys, Inc (Discussion of Commercial Products or Services). - G44
Matthew N. Newmeyer, BS - K53
National Institutes of Health, IRP, National Institute on Drug Abuse (Employee).
Thutrang Nguyen, BA - B139
Applied Biosystems, ZyGem Corporation, Ltd (Discussion of Commercial Products or Services).
Nikolaj Kjaer Nielsen - D11
Microsoft Corporation (Discussion of Commercial Products or Services).
University of Aarhus Denmark (Grant Support).
Michael S. Nirenberg, DPM - E28
Discloses no financial relationships with commercial entities.
John Nixon, CEng, MBA - D12, S2
Discloses no financial relationships with commercial entities.
Thomas J. Nolan, BA - K27
BioTage, Waters Corporation (Discussion of Commercial Products or Services).
Arcadia University (Other Financial/Material Support).
Kurt B. Nolte, MD - S1
National Institute of Justice (Grant Support).
David O. Norris, PhD - E13
Discloses no financial relationships with commercial entities.
Maher Noureddine, PhD - E21
Copan Flock Technologies, Copan Italia, Life Technologies Corporation, Smith & Wesson, The Clorox Company (Discussion of Commercial Products or Services).
Erin M. Noval, BS - E40
Cedar Crest College (Other Financial/Material Support).
Carla Miller Noziglia, MS - BS5
Universal Studios, Inc (Discussion of Commercial Products or Services).
Carolina Nuñez Vázquez, PhD - E36
Discloses no financial relationships with commercial entities.
Emilio Nuzzolese, PhD - G12
Discloses no financial relationships with commercial entities.

O

Tiffany O'Neill, DO - H105
Discloses no financial relationships with commercial entities.
Jenna L. Oakes-Smith, MFS - B206
JusticeTrax (Discussion of Commercial Products or Services).
St. Louis Metropolitan Police Department Crime Lab (Employee).
Isil Ocal - J1
Discloses no financial relationships with commercial entities.
Kathrin Ogris, MA - H6
Noras MRI Products GmbH, Siemens Corporation (Discussion of Commercial Products or Services).
Edwin O. Olaya Molina, BA - E88
Discloses no financial relationships with commercial entities.
William R. Oliver, MD - H63
National Institute of Justice (Grant Support).
Martin S. Olivier, PhD - C22
University of Pretoria and National Research Foundation, South Africa (Grant Support).
Alane Olson, MD - K75
Discloses no financial relationships with commercial entities.
Daniel Ott, PhD - B167
Collaborative Testing Services, Inc (Discussion of Commercial Products or Services).
National Institute of Standards and Technology (Employee).
Shana Ott - A58
Discloses no financial relationships with commercial entities.
Stephen D. Ousley, PhD
Jantz R.L. /Ousley, S.D. (Discussion of Commercial Products or Services) - A16, A101
National Institute of Justice (Grant Support) - A16
Erdinc Ozdemir
Discloses no financial relationships with commercial entities. - H57, K11
Sait Özsoy, MD - H99
Discloses no financial relationships with commercial entities.

P

Jacqueline L. Parai, MD - W11
Discloses no financial relationships with commercial entities.
Chan-Seong Park, PhD - D27
MIDAS Information Technology Co., Ltd (Discussion of Commercial Products or Services).
Dae-Kyoon Park, MD, PhD - E35
Discloses no financial relationships with commercial entities.
Seong Hwan Park, PhD - H14
Discloses no financial relationships with commercial entities.
Glendon Parker, PhD - B188

Lawrence Livermore National Laboratory (Paid Consultant).

Neeka M. Parker - C12
Adobe Systems Incorporated, Apple, Inc, Nikon, Inc (Discussion of Commercial Products or Services).

Walther Parson, PhD - W23
Institute of Legal Medicine, Innsbruck Medical University (Employee).

Michael N. Parsons, MS - B58
CLC bio, F. Hoffmann-La Roche, Ltd, MOBIO Laboratories, Inc, NCBI (Discussion of Commercial Products or Services).
Bode Technology (Employee).

Natascha Pascale, MD - E45
Discloses no financial relationships with commercial entities.

Ian Paul, MD - H103
Microsoft Corporation (Discussion of Commercial Products or Services).
National Institute of Justice (Grant Support).
New Mexico Office of the Medical Investigator (Employee).

Michelle R. Peace, PhD - W14
National Institute of Justice (Grant Support).
Electronic Cigarettes (Discussion of Unlabeled/Investigational Use of Product/Device).

Jennifer L. Pechal, PhD - H126
Discloses no financial relationships with commercial entities.

Michelle A. Peck, MFS
CLC bio (Discussion of Commercial Products or Services). - W23
Armed Forces DNA Identification Laboratory (Employee). - B177, W23

William K. Perdue, MPA - E44
Calumet Packaging, Foster + Freeman, Ltd, Nikon, Inc (Discussion of Commercial Products or Services).
Bureau of Alcohol, Tobacco, Firearms and Explosives (Employee).

Mark W. Perlin, PhD, MD
Cybergenetics, Inc, Life Technologies Corporation, Microsoft Corporation (Discussion of Commercial Products or Services). - B100
Cybergenetics, Inc (Discussion of Commercial Products or Services and Employee). - B100, F29

Alexis J.L. Peterson - H5
GraphPad Software, Inc (Discussion of Commercial Products or Services).
Louis Stokes Alliance for Minority Participation (Grant Support).

Lauren R. Pharr, PhD - A132
Argos GPS, ESRI, Movebank (Discussion of Commercial Products or Services).
National Science Foundation, Louisiana State University (Grant Support).

Angelina I. Phillips, MD - E29
Cessna Aircraft Company, General Dynamics Corporation (Discussion of Commercial Products or Services).

Jennifer Piel, MD, JD - I34
Discloses no financial relationships with commercial entities.

Vilma Pinchi, PhD - G11
CyberMed, Inc (Discussion of Commercial Products or Services).

Keith Pinckard, MD, PhD - H70, W3
Discloses no financial relationships with commercial entities.

Joao E.S. Pinheiro, MD - H138
Discloses no financial relationships with commercial entities.

Deborrah C. Pinto, PhD - H137
Harris County Institute of Forensic Sciences (Employee).

Dane T. Plaza, BS - B138
EMD Millipore Corporation, Life Technologies Corporation, Promega Corporation, QIAGEN, Inc (Discussion of Commercial Products or Services).

Amber M. Plemons, BS - A33
Jantz R.L./Ousley, S.D. (Discussion of Commercial Products or Services).

Christopher J. Plourd, JD - W4
Discloses no financial relationships with commercial entities.

Daniele S. Podini, PhD
Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services). - B182, E63
Promega Corporation (Discussion of Commercial Products or Services). - E63
National Institute of Justice (Grant Support). - B182
George Washington University (Employee). - E63

Justin L. Poklis, BS - W14
JEOL, Ltd, (Discussion of Commercial Products or Services).
National Institute of Justice (Grant Support).
Electronic Cigarettes (Discussion of Unlabeled/Investigational Use of Product/Device).

Adam Polhemus, BA - W14
Discloses no financial relationships with commercial entities.

Michael S. Pollanen, MD - W16
Discloses no financial relationships with commercial entities.

Mark Pollitt, PhD - W4
Discloses no financial relationships with commercial entities.

Shashank Pooniya, MD - E50
Discloses no financial relationships with commercial entities.

Amy L. Popejoy, MS - B204
Houston Forensic Science Center (Employee).

Rachel Potter, BS - K57
General Electric Company, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).
National Institute of Justice (Grant Support).

Jason Powell, MD - H132
Discloses no financial relationships with commercial entities.

Mark C. Pozzi, MS - D20, D21
Discloses no financial relationships with commercial entities.

Francesco Pradella, MSc - G52
Discloses no financial relationships with commercial entities.

Joseph A. Prahlow, MD - H69, H70
Discloses no financial relationships with commercial entities.
Western Michigan University Homer Stryker MD School of Medicine (Employee).

Samuel Prahlow - H95
Discloses no financial relationships with commercial entities.

David J. Prasek, MFS - E11
Discloses no financial relationships with commercial entities.

Sebastien S. Prat, MD - I45
Discloses no financial relationships with commercial entities.

Ulrich S. Preiß, MD - E7
Laboratoire National de Santé, Department of Legal Medicine, Dudelange, Luxembourg (Employee).

Alan A. Price, MA - S2

Discloses no financial relationships with commercial entities.

Meghan Price - A11
Boston University School of Medicine, Department of Anatomy and Neurobiology (Other Financial/Material Support).

Gregory A. Priebe, MS - K40
Shimadzu Corporation (Discussion of Commercial Products or Services).

Emily Prisaznik, BS - B21
Cedar Crest College (Other Financial/Material Support).

Ka-Man Pun
Discloses no financial relationships with commercial entities. - B14
EMD Millipore, QIAGEN, Inc, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services). - B117
University of Lausanne — School of Criminal Sciences-Institute of Forensic Sciences (Other Financial/Material Support). - B117

Matthew Pysh - D10
Discloses no financial relationships with commercial entities.

Q

Guoqiang Qian, MD - H36
Discloses no financial relationships with commercial entities.

Alicia Quinn, BS
Lee BioSolutions, Inc, Microsoft Corporation, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services). - B8
Bio-Rad Laboratories, Inc, BioFire Defense, QIAGEN, Inc (Discussion of Commercial Products or Services). - B191
TU Graduate Student Association (Grant Support). - B8, B191

R

Mithun Rajshekar, MFSc - G30
Zfx GmbH Corporation (Discussion of Commercial Products or Services).

Jed S. Rakoff, JD - S1
Discloses no financial relationships with commercial entities.

Katherine Ramsland, PhD - LW2
Discloses no financial relationships with commercial entities.

Anjali A. Ranadive, JD - F16, W8
Discloses no financial relationships with commercial entities.

Francesco Randazzo - K13
Discloses no financial relationships with commercial entities.

Sundeep S. Randhawa, MD - I25
AstraZeneca, Genetec, Inc, Pfizer, Inc (Discussion of Commercial Products or Services).

Rebekah Ranger, BA - I16
Discloses no financial relationships with commercial entities.

Anusha Rankoth - B160
Agilent Technologies, Beacon Technologies, Inc, Phenomenex, Inc (Discussion of Commercial Products or Services).

Molly M. Rathbun, BS - B178
Applied Biosystems, Clontech Laboratories, Inc, Illumina, Inc, Life Technologies Corporation (Discussion of Commercial Products or Services).
The Pennsylvania State University Forensic Science Program (Other Financial/Material Support).

Kaitlyn M. Redman, BS - B13
Applied Biosystems, Fabbrica d'Armi Pietro Beretta S.p.A., Glock, Inc, Promega Corporation, QIAGEN, Inc, Smith & Wesson, Spectronics Corperation, Taurus International MFG, Inc (Discussion of Commercial Products or Services).

Kristen L. Reese, BA - B15
Discloses no financial relationships with commercial entities.

Henry R. Reeve, JD - W4
Discloses no financial relationships with commercial entities.

Kathleen J. Reichs, PhD - BS6
Penguin Random House, FOX Broadcasting Company (Discussion of Commercial Products or Services).
FOX Broadcasting Company, Penguin Random House (Other Financial/Material Support).

Gary W. Reinecke, MA - E84
Boston University, National Institute of Justice (Grant Support).

Robin C. Reineke, PhD - A36
Colibrí Center for Human Rights (Employee).

Marcello Rendine - B26, E42
Discloses no financial relationships with commercial entities.

Thomas B. Renegar, BS - B166
Freeman Manufacturing & Supply Company, National Institute of Standards and Technology (Discussion of Commercial Products or Services).
National Institute of Standards and Technology (Employee).

Samuel R. Rennie, BSc - A26
AESOP Erasmus Mundus (Grant Support).

Jenise Reyes-Rodriguez, BS - C4
Facebook, Inc, JTAG Technologies, LinkedIn, Twitter, Inc (Discussion of Commercial Products or Services).
National Institute of Standards and Technology (Employee).

Mikaela S. Reynolds, MSc - A46
3D Systems, Inc (Discussion of Commercial Products or Services).

Im Joo Rhyu, PhD
Cybermed, Inc, Siemens Corporation (Discussion of Commercial Products or Services). - A107
Discloses no financial relationships with commercial entities. - H14

Pietrantonio Ricci - E10, H97, H98
Discloses no financial relationships with commercial entities.

Charles A. Richardson-Gongora - B119
Discloses no financial relationships with commercial entities.

Jason D. Ricke, JD, LLM - F37
Harris Corporation (Discussion of Commercial Products or Services).

Anders Rietz - E1
Discloses no financial relationships with commercial entities.

George R. Riley, PhD - B116
National Center for Biotechnology Information, National Institute of Health, National Library of Medicine (Discussion of Commercial Products or Services).
National Institute of Health (Employee).

Sarah Riman, PhD - B182
George Washington University (Other Financial/Material Support).

Joseph D. Ring, MS
Advanced Analytical Technologies, Inc, Agilent Technologies, Beckman Coulter, Inc, CLC bio, Clontech Laboratories, Inc, Hamilton Company, Illumina, Inc, Integrated DNA Technologies, Inc, KAPA Biosystems, New England BioLabs, QIAGEN, Inc, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services). - B177
Advanced Analytical Technologies, Inc, Agilent Technologies, Hamilton Company, KAPA Biosystems, QIAGEN, Inc (Discussion of Commercial Products or Services). - W23
Armed Forces DNA Identification Laboratory (Employee). - B177, W23
Irma Rios, MBA - E62
National Institute of Justice (Grant Support).
Jariangely Rivera - H23
Matchett, A. (Other Financial/Material Support).
Graham J. Roberts, MDS - G4
Discloses no financial relationships with commercial entities.
Lindsey G. Roberts, MA - A20
Southern Illinois University (Employee).
Brianna L. Robinson - A17
Rice Creek Associates Small Grants Program (Grant Support).
Nancy Rodriguez, PhD - S1
National Institute of Justice (Employee).
Sandra E. Rodriguez-Cruz, PhD - B90, B118
Department of Justice (Employee).
Scott Roeske, MFS - W7
Discloses no financial relationships with commercial entities.
Marcus Rogers, PhD
Google, Inc, Microsoft Corporation (Discussion of Commercial Products or Services). - C7
Discloses no financial relationships with commercial entities. – W22
Meghan Roig, BS - H20
Pressure BioScience, Inc (Discussion of Commercial Products or Services).
Florida International University, Department of Chemistry and Biochemistry (Other Financial/Material Support).
Christopher M. Rollman, BS - B49
National Science Foundation (Grant Support).
Amelia Romoser, PhD - K70
Discloses no financial relationships with commercial entities.
Erica L. Romsos, MFS - B215
Promega Corporation, QIAGEN, Inc, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).
National Institute of Standards and Technology Law Enforcement Standards Office (Employee).
Jeri D. Ropero-Miller, PhD
The National Institute of Justice (Grant Support). - S1
The National Institute of Justice Forensic Technology Center of Excellence (Employee). - W12
Roberto Rosa, PhD - B164
Princeton Applied Research (Discussion of Commercial Products or Services).
Karen B. Rosenbaum, MD - I6
Discloses no financial relationships with commercial entities.
Adam R. Rosenblatt, PhD - A37
Mellon/American Council of Learned Societies Dissertation Completion Fellowship (Grant Support).
Alastair Ross, AM - W9
Discloses no financial relationships with commercial entities.
Ann H. Ross, PhD - A3
Jantz R.L./Ousley, S.D. (Discussion of Commercial Products or Services).
Walter F. Rowe, PhD - B195
Thermo Fisher Scientific, Inc, IBM Corporation (Discussion of Commercial Products or Services).
George Washington University (Employee).
Katie M. Rubin, MS - A87
H. David Sheets, Immersion Corporation (Discussion of Commercial Products or Services).
Norah Rudin, PhD - B174
SCIEG (Discussion of Commercial Products or Services).
Eric R. Ruiz Hernandez, MD - E49, E52
Discloses no financial relationships with commercial entities.
Stewart D. Ryckman, MD - W24
Discloses no financial relationships with commercial entities.

S

Kenneth J. Saczalski, PhD
Independent Supported Research (Paid Consultant). - D2
Discloses no financial relationships with commercial entities. - D4
Anthony J. Saitta - B9
QIAGEN, Inc (Discussion of Commercial Products or Services and Discussion of Unlabeled/Investigational Use of Product/Device).
Summer Undergrad Research Fellowship, SURF, University of New Haven (Other Financial/Material Support).
Michael J. Saks - F20
Discloses no financial relationships with commercial entities.
Warren C. Samms, PhD - B31
Harris County Institute of Forensic Sciences (Employee).
Isidora Samojlik, MD, PhD - K14
The Ministry of Education and Science of the Republic of Serbia (Grant Support).
University of Novi Sad, Faculty of Medicine (Employee).
Michelle R. Sanford, PhD - H116
Harris County (Employee).
Robert M. Sanger, JD - F4
Discloses no financial relationships with commercial entities.
Alora Sansola - H84
IriTech, Inc (Discussion of Commercial Products or Services).
Sierra Santana, BA - A12
Discloses no financial relationships with commercial entities.
Kelly Sauerwein, MA - B18
University of Tennessee (Employee).
Tiffany B. Saul, MS - B18
University of Tennessee (Employee).
Melanie A. Schade - B19
Cedar Crest College (Other Financial/Material Support).
Maureen Schaefer, PhD - A54
Michigan State University (Employee).
Sarah Schaerli - H90

Siemens Medical Solutions USA, Inc (Discussion of Commercial Products or Services).

Jairo G. Schafer, MSc - F11
Discloses no financial relationships with commercial entities.

Jason E. Schaff, PhD - W2
Agilent Technologies, SCIEX, Waters Corporation (Discussion of Commercial Products or Services).

William C. Schaffer, MA - A108
Discloses no financial relationships with commercial entities.

Eileen M. Schilling, MSc - A41
Discloses no financial relationships with commercial entities.

Jennifer R. Schindell, MA - E48
Discloses no financial relationships with commercial entities.

Tyler J. Schlagetter - B6
University of New Haven (Employee).

Carl J. Schmidt, MD - H74
IBM Corporation, Microsoft Corporation (Discussion of Commercial Products or Services).
University of Michigan (Employee).

Howard A. Schmidt, MS - S1
Discloses no financial relationships with commercial entities.

Candace H. Schoppe, MD - W17
Discloses no financial relationships with commercial entities.

Jason L. Schroeder, MS, MBA - E54
Discloses no financial relationships with commercial entities.

Ellen M. Schuetzner, BA - W6
National Institute of Justice (Grant Support).

Daniel L. Schultz, MD - H53
Discloses no financial relationships with commercial entities.

John J. Schultz, PhD - A80
Discloses no financial relationships with commercial entities.

David M. Schwope, PhD - W2
Aegis Scientific, Inc, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).
Aegis Labs (Employee).

Gary T. Scott, MA
Discloses no financial relationships with commercial entities. - A18
Metropolitan State University of Denver (Employee) - A58

Veronica Scotti, JD - F19
Discloses no financial relationships with commercial entities.

Sarah J. Seashols Williams, PhD - B53
Life Technologies Corporation, QIAGEN, Inc (Discussion of Commercial Products or Services).
Jeffress Foundation (Grant Support).

Ismail M. Sebetan, MD, PhD
EMD Millipore Corporation, Life Technologies Corporation, MACHEREY-NAGEL GmbH & CO KG, Santorius Stedim Biotech, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services). - B133
Paternity Testing Corporation, QIAGEN, Inc, Tecan Schweiz AG, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services). - B134
Discloses no financial relationships with commercial entities. - A113, E11, F10

Season E. Seferyn, MSFS - E68
National Institute of Justice (Discussion of Commercial Products or Services).

Andrew C. Seidel, MA - A94
Discloses no financial relationships with commercial entities.

Kathryn C. Seigfried-Spellar, PhD
Amazon.com, Inc, Qualtrics, LLC (Discussion of Commercial Products or Services). - C3
Discloses no financial relationships with commercial entities. - W22

Joshua Seither, MS - K47
Discloses no financial relationships with commercial entities.

David R. Senn, DDS - G32
Discloses no financial relationships with commercial entities.

Ayse Serin, PhD
Cukurova University (Grant Support) - H11
Discloses no financial relationships with commercial entities. - H15

Serenella Serinelli, MD - H101
Discloses no financial relationships with commercial entities.

Javier G. Serrano, MD - H85
Puerto Rico Institute of Forensic Sciences (Employee).

Francesco Sessa, MS - A102
Discloses no financial relationships with commercial entities.

Heather J. Seubert, MS - B92
Alicona Imaging GmbH, ScanBi Technology, Sensofar, Topmatch (Discussion of Commercial Products or Services).

Krishna D. Shah, MD - H77
Discloses no financial relationships with commercial entities.

John P. Shand, MD - I34
Discloses no financial relationships with commercial entities.

Piyush Sharma, MD - A66
All India Institute of Medical Sciences (Employee).

Donald E. Shelton, JD, PhD - F14, F22
Discloses no financial relationships with commercial entities.

Claire E. Shepard, MS - S2
Discloses no financial relationships with commercial entities.

Mary Shields, DMD - G35
Discloses no financial relationships with commercial entities.

Sang Eon Shin - H14
The Ministry of Education, Science and Technology (Speakers Bureau).

Elisa N. Shoff, BS - K67
Bruker Corporation, Thermo Fisher Scientific, Inc, UCT, Inc (Discussion of Commercial Products or Services).
Miami-Dade Medical Examiner Department (Employee).

Mark J. Shuman, MD - H24
Discloses no financial relationships with commercial entities.

Inga Siebke - A97
Discloses no financial relationships with commercial entities.

Michael E. Sigman, PhD - B171
National Institute of Justice (Grant Support).
University of Central Florida (Employee).

William E. Silver, DDS - G54
Discloses no financial relationships with commercial entities.

Tal Simmons, PhD - A59, A119
Discloses no financial relationships with commercial entities.

Terrie Simmons-Ehrhardt, MA - E82
Information Science and Technologies Institute - CNR, Kitware, Inc, Materialise, National Cancer Institute, SAS IP, Inc (Discussion of Commercial Products or Services).
Virginia Commonwealth University (Employee).
National Institutes of Justice (Grant Support).

Alison Simon, BS - B27
Discloses no financial relationships with commercial entities.

Rachel S. Singer, JD - F26
Discloses no financial relationships with commercial entities.
Ankit Kumar Singh, BS - C23
Auburn Cyber Research Center (Other Financial/Material Support).
Baneshwar Singh, PhD
InnoGenomics Technologies, LLC, Life Technologies Corporation, QIAGEN, Inc (Discussion of Commercial Products or Services) - B145
Virginia Commonwealth University (Grant Support) - B145
Free Software Foundation, Inc, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services). - H117
National Institute of Justice, Virginia Commonwealth University (Grant Support). - H117
Pankaj Sinha - K35
Randox Toxicology, Ltd (Employee).
Kitrina D. Skaggs, BA - B80
Discloses no financial relationships with commercial entities.
Cassie E. Skipper, BS - A10
Discloses no financial relationships with commercial entities.
Anthony Skjellum, PhD
Google, Inc (Discussion of Commercial Products or Services). - C18
Auburn Cyber Research Center (Other Financial/Material Support). - C23
Discloses no financial relationships with commercial entities. – W22
Catyana R. Skory Falsetti, MFS - F18
Google, Inc, Maxon Computer, Inc, Microsoft Corporation (Discussion of Commercial Products or Services).
Maricopa County Attorney's Office (Employee).
Donia Slack, MS - B103
CLC bio, Illumina, Inc, Promega Corporation, QIAGEN, Inc (Discussion of Commercial Products or Services).
CTTSO/TSWG, Bode Cellmark Forensics (Grant Support).
Kathryn Sloper, BS - A112
Discloses no financial relationships with commercial entities.
Jeff M. Smith, MS - C14
GoPro, Inc (Discussion of Commercial Products or Services).
Lauren R. Smith, BS - H8
Illumina, Inc, Knight Lab (Discussion of Commercial Products or Services).
National Institute of Justice, Sam Houston State University (Grant Support).
Vivian Snyder, DO - W3
Discloses no financial relationships with commercial entities.
Tore T. Solheim - G46
Plass Data Software A/S (Discussion of Commercial Products or Services and Discussion of Unlabeled/Investigational Use of Product/Device).
April D. Solomon, BS - B108
EMD Millipore Corporation, PerkinElmer, Inc, QIAGEN, Inc, STRATEC Biomedical AG, Thermo Fisher Scientific, Inc, ZyGem Corporation, Ltd (Discussion of Commercial Products or Services).
National Institute of Justice (Grant Support).
Junfeng J. Song, MS - B169
National Institute of Standards and Technology (Employee).
Amy E. Sorensen, MSFS - B113
Biomatrica, Inc, DNA Genotek, Inc, Life Technologies Corporation, Promega Corporation (Discussion of Commercial Products or Services).
Sam Houston State University (Employee).
Miriam E. Soto Martinez, MA - A103
Texas Center for the Judiciary - Children's Justice Act (Grant Support).
Harris County Institute of Forensic Sciences (Employee).
Curtis E. Sparling, MA - W7
U.S. Army CID (Employee).
Debi Spencer, MFS - W24
Discloses no financial relationships with commercial entities.
Kate Spradley, PhD - A5
Texas State University (Employee).
Susan Sprogoe-Jakobsen - H81
Discloses no financial relationships with commercial entities.
Cristina E. Stanciu, BS - B106
Becton, Dickinson and Company (Discussion of Commercial Products or Services).
Virginia Commonwealth University (Employee).
Dawnie W. Steadman, PhD - A128
University of Tennessee — National Institute of Justice (Grant Support).
Paul Stein, PhD
EMD Millipore Corporation, Life Technologies Corporation, MACHEREY-NAGEL GmbH & CO KG, Santorius Stedim Biotech, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services). - B133
Paternity Testing Corporation, QIAGEN, Inc, Tecan Schweiz AG, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services). - B134
Discloses no financial relationships with commercial entities. - E11, F10
Carl N. Stephan, PhD - A70
Phil Harvey (Discussion of Commercial Products or Services).
The University of Queensland (Employee).
Peter J. Stephens, MD - LW4
Discloses no financial relationships with commercial entities.
Jennifer L. Stephenson, MSFS - E38
Remington Arms Company, LLC, Sensofar, Sturm, Ruger & Co., Inc (Discussion of Commercial Products or Services).
Babatunde L. Stokes, MD - H33
Discloses no financial relationships with commercial entities.
Nancy A. Stokes, MS - B105
Promega Corporation (Discussion of Commercial Products or Services).
Mark D. Stolorow, MS, MBA
Discloses no financial relationships with commercial entities. - B41
National Institute of Standards and Technology (Employee). - BS3
Joseph Stone, BS - K7
JEOL, Ltd, (Discussion of Commercial Products or Services).
National Institute on Health Center for Drug Abuse (Grant Support).
David A. Stoney, PhD
Sirchie Finger Print Laboratories (Discussion of Commercial Products or Services). - B85

National Institute of Justice (Grant Support). - B85
Stoney Forensic, Inc (Employee). - F38

Michael P. Stypa, MS - K50
Las Vegas Metropolitan Police Department (Employee).

Vanessa Sufrin, MS - H117
Free Software Foundation, Inc, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).
National Institute of Justice, Virginia Commonwealth University (Grant Support).

Garett Sugimoto, MS - B100
Cybergenetics, Inc, Life Technologies Corporation, Microsoft Corporation (Discussion of Commercial Products or Services).
Kern Regional Crime Laboratory (Employee).

Mary Sullivan, MSN - I20
Discloses no financial relationships with commercial entities.

Travis M. Sullivan, BS - H44
Discloses no financial relationships with commercial entities.

Andrew Sulner, MSFS, JD - F23, F47
Discloses no financial relationships with commercial entities.

Joel D. Sutton, MSFS - B174
NicheVision, Inc (Discussion of Commercial Products or Services).
U.S. Army CIL/DFFS (Employee).

Henry J. Swofford, MSFS - B161, F17
U.S. Army CIL (Employee).

T

Yoshitaka Takase, MS - C7
Discloses no financial relationships with commercial entities.

Fredrik Tamsen, MD, MSc - E3
The Swedish National Board of Forensic Medicine (Employee).

Tobin A. Tanaka, BS - J17
Discloses no financial relationships with commercial entities.

Melissa K. Taylor, BA - W1
National Institute of Standards and Technology (Discussion of Commercial Products or Services and Employee).

Tammy Taylor, MS - B209
Life Technologies Corporation (Discussion of Commercial Products or Services).
Harris County Institute of Forensic Sciences (Employee).

Jeff Teitelbaum, MS - W1
Google, Inc, National Center for Biotechnology Information, OCLC Online Computer Library Center, Inc, U.S. Department of Justice (Discussion of Commercial Products or Services).
Washington State Patrol (Employee).

Silvana Temi, MD - H40
Discloses no financial relationships with commercial entities.

Keith-Dane H. Temporal, BS - K61
Waters Corporation (Discussion of Commercial Products or Services).

MariaTeresa A. Tersigni-Tarrant, PhD - A100, H80
Discloses no financial relationships with commercial entities.

Patrick W. Thevissen, PhD - G7
Discloses no financial relationships with commercial entities.

Rebecca Thielen, BS - B39
Agilent Technologies, Bridgestone Manufacturing Company, Faulkin Tire, GITI Tire Pte., Ltd, Michelin Manufacturing Company, The Goodyear Tire & Rubber Company, (Discussion of Commercial Products or Services).

Phoutthasone Thirakul, MD - H53
Discloses no financial relationships with commercial entities.

Brittany Thomas, MFS
AIT Laboratories, NMS Labs (Discussion of Commercial Products or Services). - K45
Washington State Patrol Toxicology Laboratory (Employee). - K1, K45

Sara R. Thomas, MS - I42
Discloses no financial relationships with commercial entities.

Monica M. Thompson - A91
Discloses no financial relationships with commercial entities.

Robert M. Thompson, BS
Discloses no financial relationships with commercial entities. - B169, W20
Alicona Imaging, GmbH, GelSight, Inc, NanoFocus AG, Leica Microsystems, Sensofar (Discussion of Commercial Products or Services). - B173
National Institute of Standards and Technology (Employee). - B173

Ronald R. Thrasher, PhD - I9
Discloses no financial relationships with commercial entities.

Morris V. Tidball-Binz, MD - W16
Discloses no financial relationships with commercial entities.

Andreas Tillmar, PhD - B208
Discloses no financial relationships with commercial entities.

Meredith L. Tise, PhD - A89
SAS Institute, Inc, Solution Technologies, Inc (Discussion of Commercial Products or Services).

Cathy Tobin - W19
Discloses no financial relationships with commercial entities.

Kyle Tom, MS - B163
Thomson Reuters (Discussion of Commercial Products or Services).
Federal Bureau of Investigation (Employee).

Patrizia Trapella, JD, MA - E 85, F1
Discloses no financial relationships with commercial entities.

Lauren Traveller, DNP - F46
Discloses no financial relationships with commercial entities.

Giuseppe Troccoli, MD - I20
Discloses no financial relationships with commercial entities.

Janamarie Truesdell, MSc
Eley, K. (Discussion of Commercial Products or Services) - A48
Discloses no financial relationships with commercial entities. - A83

Marcia Aiko Tsunoda, Msc - F11
Brazilian Federal Police (Employee).

Lucile Tuchtan, MD - D28, E43
Discloses no financial relationships with commercial entities.

Hugh H. Tuller, MA - A35
Defense POW/MIA Accounting Agency (Employee).

Nilesh K. Tumram, MD - H52, H136
Discloses no financial relationships with commercial entities.

Nursen Turan, MD - I41
Discloses no financial relationships with commercial entities.

Katherine Turner, BS - K3
Cuyahoga County Medical Examiner's Office (Discussion of

Commercial Products or Services).
Nichole M. Tuscher, MFS - B133
EMD Millipore Corporation, Life Technologies Corporation, MACHEREY-NAGEL GmbH & CO KG, Santorius Stedim Biotech, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).
Marykathryn Tynon, MSFS - K59, K64
Waters Corporation (Discussion of Commercial Products or Services).
NMS Labs (Employee).

U

Douglas H. Ubelaker, PhD - W16
Discloses no financial relationships with commercial entities.
Naeem Ullah, BS - W5
Office of Chief Medical Examiner City of New York (Discussion of Commercial Products or Services and Employee).
Ayca Ulubay - H15
Applied Biosystems, Life Technologies Corporation (Discussion of Commercial Products or Services).
Cukurova University Department of Forensic Medicine (Employee).
Noelle J. Umback, PhD - S2, W8
Discloses no financial relationships with commercial entities.
Esra Unal, MD - I4, I13
Discloses no financial relationships with commercial entities.
Petra Urbanová, PhD - C21
Berkeley Vision and Learning Center, Canfield Scientific, Inc, Fidentis (Discussion of Commercial Products or Services).
Masaryk University (Employee).
Abdullah Usman, LLM, MSc - F44
Discloses no financial relationships with commercial entities.
Yuriy Uvaydov, MS - B75
IonSense, Inc, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).
Drug Enforcement Administration (Employee).

V

Julie L. Valentine, MS - E56
Brigham Young University (Employee).
Peter M. Vallone, PhD - W23
National Institute of Standards and Technology (Employee).
Arian C. van Asten, PhD
Netherlands Forensic Institute (Employee). - D17, W20
Netherlands Organization for Scientific Research (Grant Support). - D17
Lisa M.M. Van Den Broek - A45
Information Science and Technologies Institute - CNR (Discussion of Commercial Products or Services).
Victor Vandell, PhD - K31
Agilent Technologies, BioTage, SCIEX (Discussion of Commercial Products or Services).
BioTage (Employee).
Stefano Vanin, PhD - G36, H93
Discloses no financial relationships with commercial entities.
Nancy Vargas Becerril, PhD - G3
Discloses no financial relationships with commercial entities.
Thomas W. Vastrick, BS
Discloses no financial relationships with commercial entities. J5, J19
National Institute of Justice (Grant Support). - W6
Patrick E. Vaughan, BS - D3
Michigan State University Engineering EnSURE Program, Orthopaedic Biomechanics Laboratories (Other Financial/Material Support).
Jessica Ann Veltri, MS - W7
U.S. Army CID (Employee).
Elvira Ventura Spagnolo - E5
Discloses no financial relationships with commercial entities.
Athina Vidaki, PhD - B192
Illumina, Inc (Discussion of Commercial Products or Services).
Papadaki Foundation, National and Kapodistrian University of Athens King's College London, University of London (Grant Support).
Duarte Nuno Vieira, MSc, PhD, MD
Discloses no financial relationships with commercial entities. - W16
Faculty of Medicine - University of Coimbra (Employee). - E83
Margarita M. Villarreal, BS - A109
Discloses no financial relationships with commercial entities.
AnniLauri Villeme, BS - B142
Future Technologies, Inc, InnoGenomics Technologies, LLC (Discussion of Commercial Products or Services).
U.S. National Institute of Standards and Technology - Guest Researcher (Other Financial/Material Support).
Mark D. Viner, MSc - W18
Cranfield University (Employee).
Kyle E. Vircks, MS - B199
JEOL, Ltd, (Discussion of Commercial Products or Services)
National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. (Grant Support).
Silvia D. Visonà, MD - H38
Discloses no financial relationships with commercial entities.
Giulia Vitale - G10
Discloses no financial relationships with commercial entities.
Erin L. Vollmer, BA - B3
Applied Biosystems, EMD Millipore Corporation, QIAGEN, Inc (Discussion of Commercial Products or Services).
Jennifer M. Vollner, MS - A76
Michigan State University (Employee).
Laura Volpini, PhD - I43
Discloses no financial relationships with commercial entities.

W

Erin Waddell, PhD - B154
3M, Agilent Technologies, Waters Corporation (Discussion of Commercial Products or Services).
Visiting Scientist Program at the FBI, Oak Ridge Institute for Science and Education (Other Financial/Material Support).
Ruth Waddell Smith, PhD - B46
Michigan State University (Employee).
Audriana M. Wagner - B38

Discloses no financial relationships with commercial entities.

Sarah Wagner - A34
Discloses no financial relationships with commercial entities.

Crystal L. Wagoner, MFS - E69
Discloses no financial relationships with commercial entities.

Erin Walsh - K39
Discloses no financial relationships with commercial entities.

Richard D. Walter, MA - W21
Discloses no financial relationships with commercial entities.

Heather E. Waltke, MS - B213
National Institute of Justice (Employee).

John Z. Wang, PhD - E27
Discloses no financial relationships with commercial entities.

Ling Wang, MS - B36
National Institute of Justice (Grant Support).

Wego Wang, SciD - D6
Discloses no financial relationships with commercial entities.

Wendy S. Warren, DO - H49
Discloses no financial relationships with commercial entities.

Steven B. Watson, BA - W15
Elma Schmidbauer GmbH (Discussion of Commercial Products or Services).

Daniel Watsula, MS - B115
Bode Cellmark Forensics, Life Technologies Corporation, QIAGEN, Inc (Discussion of Commercial Products or Services).
Bode Cellmark Forensics (Employee).

Jalika Rivera Waugh, PhD - E9
Discloses no financial relationships with commercial entities.

Courtney Weatherbee, BS – H96
Discloses no financial relationships with commercial entities.

Ingrid T. Weber, PhD - B158
CBC, Glock, Inc (Discussion of Commercial Products or Services).

Victor W. Weedn, MD, JD - W20
George Washington University (Employee).

Robert Weinstock, MD - I6, I40
Discloses no financial relationships with commercial entities.

Kelsie R. Weir, BA - B1
Applied Biosystems, BioTek Instruments, Inc, Minitab, Inc, QIAGEN, Inc, Spectronics Corperation, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).
University of New Haven (Other Financial/Material Support).

Kurt D. Weiss, MS
Discloses no financial relationships with commercial entities. - D18
Racelogic, United Kingdom (Discussion of Commercial Products or Services). - D22

Jeffrey D. Wells, PhD - H119
National Institute of Justice (Grant Support).

Daniel J. Wescott, PhD - A60, A117
Texas State University (Employee).

Roland Wessling, MSc - A47
Discloses no financial relationships with commercial entities.

Christian G. Westring, PhD - BS3
NMS Labs (Employee).

Amanda Wheeler, BS - B109
QIAGEN, Inc, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).

Douglas R. White, MS - F48
National Institute of Standards and Technology, U.S. Department of Commerce (Discussion of Commercial Products or Services).
National Institute of Standards and Technology (Employee).

Joseph L. White, MS - C8
Cellebrite, Guidance Software, Inc, Snapchat, Inc (Discussion of Commercial Products or Services).
U.S. Army CIL (Employee).

Jason M. Wiersema, PhD
Discloses no financial relationships with commercial entities. - H78
Harris County Institute of Forensic Sciences (Employee) - E8

Matthew C. Wietbrock, BS - E24, E46
Discloses no financial relationships with commercial entities.

Kelsey L. Wilkinson, BS - C9
Apple, Inc, Cellebrite, Google, Inc, MSAB, The Raspberry Pi Foundation (Discussion of Commercial Products or Services).
Marshall University (Grant Support).

Amanda Williams, MA - A52
UNR Graduate Student Association Research Grant and Alice M. Brues Research Award (Grant Support).

Chinyere M. Williams, BS - W19
Discloses no financial relationships with commercial entities.

John A. Williams, PhD - S2
Discloses no financial relationships with commercial entities.

Mary R. Williams, MS - B150
National Institute of Justice-Office of Justice Programs, University of Central Florida. (Grant Support).

Tyler Williams - B121
Agilent Technologies, Cerilliant Corporation (Discussion of Commercial Products or Services).

Sheila Willis, PhD - W9
Discloses no financial relationships with commercial entities.

Emily K. Wilson - A125
Defense POW/MIA Accounting Agency (Employee).
Oak Ridge Institute for Science and Education (Other Financial/Material Support).

Laura A. Wilson, BS - B175
Illumina, Inc, Life Technologies Corporation, Takara Bio, Inc (Discussion of Commercial Products or Services).
Penn State University (Other Financial/Material Support).

Mark R. Wilson, PhD - B101
Cybergenetics, Inc (Discussion of Commercial Products or Services).
Western Carolina University (Employee).
Probabilistic Software for DNA Mixture Deconvolution (Discussion of Unlabeled/Investigational Use of Product/Device).

Shannon Wilson - D8
Eos Systems, Inc, Faro Technologies, Inc, Trimble Navigation, Ltd (Discussion of Commercial Products or Services).

Jessica Winborn, BS - B78
National Institute of Justice, Office of Justice Programs, U.S. Department of Justice (Grant Support).

Gwyn Winfield, MA - W20

Falcon Communications (Employee).

Barbara C. Wolf, MD - W10
CRC Press, LLC (Discussion of Commercial Products or Services).

Carl E. Wolf II, PhD - K71
BioTage, Restek Corporation, Waters Corporation (Discussion of Commercial Products or Services).

Eun Jin Woo, PhD - A44
GOM, VATECH America, Z Corporation (Discussion of Commercial Products or Services).

Matthew R. Wood, MS
NSF-CRIF and the Rutgers Academic Excellence Fund (Grant Support). - B124
Discloses no financial relationships with commercial entities. - W14

Robert E. Wood, DDS, PhD - G50, G51
Discloses no financial relationships with commercial entities.

Michael S. Woolf, BS
Discloses no financial relationships with commercial entities. - A59
Free Software Foundation, Inc, Illumina, Inc, The R Foundation, UCHIME (Discussion of Commercial Products or Services). - H10

Sharon C. Wootton, PhD - B176
Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services and Employee).

Charlotte J. Word, PhD - B214, F30
Discloses no financial relationships with commercial entities.

Diana M. Wright, PhD - B87
Department of Justice (Employee).

Y

Chu-An Yang, MS - K32
Agilent Technologies (Discussion of Commercial Products or Services).

Tiantong Yang - H36, H65, H141
Discloses no financial relationships with commercial entities.

Zhiyong Yang, MD - H36
Discloses no financial relationships with commercial entities.

Jillian K. Yeakel, MS - W2
Agilent Technologies (Discussion of Commercial Products or Services).
Lehigh Valley Toxicology (Employee).

Seija Ylijoki-Sørensen, MD, DDS, PhD - E72
Discloses no financial relationships with commercial entities.

Tanasiri Yokchue, MSc - K37
Royal Thai Government (Grant Support).

Seong Ho Yoo, PhD - H14
Discloses no financial relationships with commercial entities.

John L. Young, MD - I7
American Psychiatric Publishing (Discussion of Commercial Products or Services).

Z

Elazar Zadok - E55
Discloses no financial relationships with commercial entities.

Andrea Zaferes, BA - W10
Discloses no financial relationships with commercial entities.

Sara C. Zapico, PhD - A85
Discloses no financial relationships with commercial entities.

Wolf-Dieter Zech, MD - H131
Discloses no financial relationships with commercial entities.

Kathryn A. Zegarelli, BS - E39
Boston University (Employee).
National Institute of Justice Award (Grant Support).

Sharon E. Zeller, BS - B145
InnoGenomics Technologies, LLC, QIAGEN, Inc, Thermo Fisher Scientific, Inc (Discussion of Commercial Products or Services).
Virginia Commonwealth University (Grant Support).

Xiang Zhang, MD
Discloses no financial relationships with commercial entities. - H36, H141
Office of the Chief Medical Examiner, State of MD (Employee). - H65

Xiaoyu A. Zheng, MS - B168
Fabbrica d'Armi Pietro Beretta S.p.A., Sturm, Ruger & Co., Inc (Discussion of Commercial Products or Services).
National Institute of Standards and Technology (Employee).

Lawrence Ziegler, PhD - B184
Boston University, National Institute of Justice (Grant Support and Employee).

Lawrence Ziegler, PhD - E39
National Institute of Justice (Grant Support).

Patrick Zirpoli - W21
Discloses no financial relationships with commercial entities.

Joel A. Zlotnick, MSFS
Adobe Systems Incorporated, Foster + Freeman, Ltd, GIMP, Regula, Sony Corporation of America, Ultra Electronics Forensic Technology (Discussion of Commercial Products or Services) - J6
U.S. Department of State, Bureau of Consular Affairs (Employee). - J6, J13. J17

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PRESENTING AUTHOR INDEX

The presenting author index can provide a quick reference to find when and in what section presenting authors are scheduled to present at the 2016 Annual Scientific Meeting. The reference table below assists you in finding the section in which the presentation will be given. Letters correspond to the scientific discipline/section in which the presentation is being made while the number corresponds to the numerical sequence of the presentation within the section.

A	Anthropology	J	Questioned Documents
B	Criminalistics	K	Toxicology
C	Digital & Multimedia Sciences	LW	Last Word Society
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